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Specification and Drawings, as originally filed, with Application for Patent Serial No: **2,230,201**, on April 27, 1998, by **HSC RESEARCH AND DEVELOPMENT LIMITED PARTNERSHIP,** assignee of Sean E. Egan, Wei Wang and Ameet Sengar, for "Ese Genes and Proteins".

# PRIORITY DOCUMENT

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Date

# ESE GENES AND PROTEINS

## Field of the Invention

This invention relates to novel mammalian proteins identified as Ese1 and Ese2 which are involved in endocytosis via clathrin coated pits.

## Background of the Invention

Endocytosis via clathrin-coated pits is a multipstep process (1, 2). Clathrin heavy and light chains are brought to the plasma membrane through association with a heterotetrameric complex known as clathrin adaptor complex 2, or AP-2. At coated pits, the membrane is bent through the assembly of clathrin triskelion into a caged lattice. The GTPase Dynamin is also recruited to the neck of coated pits where it assembles into a collar for vesicle fission (4, 5). Recruitment of Dynamin to coated pits is believed to require the Amphiphysin I/II heterodimer (6), as these proteins bind Dynamin *in vivo* and amphiphysin proteins are required for endocytosis in yeast (7). In addition, ectopic expression of either Amphiphysin I or II by themselves (6), or the isolated SH3 domain of Amphiphysin I blocks endocytosis (8, 9). Recent data has revealed that the Rab5 small GTPase is required for sequestration of ligands such as transferrin and Epidermal Growth Factor into coated pits *in vitro* (10). The mechanism by which these components interact to regulate coated pit assembly, cargo sequestration, followed by vesicle fission is not yet understood.

From biochemical, cell biological and genetic analysis it is clear that additional components such as kinases, phosphatases, ubiquitin conjugating enzymes as well as lipid modifying enzymes are required for clathrin-coat and vesicle formation (1, 11, 12, 13, 14, 15, 16, 17, 18, 19). Indeed there is also strong evidence for a requirement of the actin cytoskeleton in endocytosis and several proteins which may facilitate this connection (1, 7, 20, 21, 22, 23, 24, 25).

The Eps15 protein was discovered in a search for substrates of the Epidermal Growth Factor Receptor(26). In 1995, Benmerah et. al. reported that Eps15 is constitutively associated with α-adaptin of the AP2 complex (27). The Eps15 protein has also been localized to the neck of clathrin-coated pits by immunoelectron microscopy (28, 29). Recently, two groups have used dominant inhibitory mutants of Eps15, or antibodies against Eps15 (or the related protein Eps15R), to demonstrate that Eps15 proteins are required for endocytosis via clathrin-coated pits (30, 31). Eps15 contains three large

structural domains(26, 32). The N-terminal third contains three copies of an EH domain (for Eps15 Homology domain) (32, 33). The central region of Eps15 forms an extended coiled-coil, which is followed by a complex C-terminus containing SH3-binding motifs (34), a large number of DPF repeats (Aspartic acid-Proline-Phenylalanine) of unknown function, and α-adaptin binding sequences (31, 35, 36). The full length Eps15R protein has a similar overall organization (34). Both Eps15 and Eps15R can be alternatively spliced to produce numerous smaller proteins(37).

A protein with similar overall organization has been identified *in Saccharomyces cerevisiae* named Pan1p. Genetic analysis of *PAN1* has revealed that this gene is required for endocytosis and for organization of the actin cytoskeleton (23, 38). Like Eps15 and Eps15R in mammals, the Pan1p protein has N-terminal EH domains followed by a central coiled-coil domain and C-terminal proline-rich sequences. A second EH domain containing protein, End3p, has also been described in *S. cerevisiae* which is required for endocytosis and regulation of the actin cytoskeleton (21, 39). Co-immunoprecipitation studies have shown that Pan1p and End3p form a complex *in vivo* (40) . Indeed, overexpression of End3p can suppress the phenotype of *pan1-4* hypomorphic mutants, and Pan1p is mislocalized in *end3* mutants indicating that these proteins function together (40). Additional studies have revealed that the EH domains of Pan1p bind to yeast homologues of mammalian clathrin-binding proteins, AP180 and CALM (yAP180A and yAP180B), through NPF motifs (Asparagine-Proline-Phenylalanine) in the yAP180 C-termini(25). These data have led to a proposal that the Pan1p:End3p complex functions as a multivalent adaptor to coordinate protein-protein interactions during endocytosis (25, 40). At least two additional proteins are predicted to bind to the Pan1p:End3p complex *in vivo*, as strong genetic interactions have been detected between PAN1 and SJL1(25), and between PAN1 and RSP5 (41). SJL1 encodes a phosphatidylinositol polyphosphate-5-phosphatase protein which is related to mammalian synaptojanin (42) and has a C-terminal NPF motif predicted to bind to EH domains in Pan1p (or End3p) (25, 43) . RSP5 encodes an E3 ubiquitin-protein ligase which may bind to the C-terminal polyproline sequences in Pan1p through one of its three WW domains (25).

Numerous SH3 domain containing proteins have been implicated in the regulation of endocytosis (44). These include Amphiphysin I(45) and II (6, 46, 47, 48), Rsv161/Rsv167(7), Actin Binding Protein-1(49), Endophilin/SH3P4/8/13 (50, 51) and Grb2 (52). Kay and coworkers have reported the isolation of several novel SH3 encoding cDNAs (53).

The present inventors have identified novel mammalian proteins containing both EH and SH3 domains, which have been named Ese1 and Ese2. Sequence and functional analysis of the full length proteins have implicated these proteins in both pinocytosis and receptor mediated endocytosis via clathrin coated pits and therefore the proteins have been named Ese1 and Ese2 respectively (Ese: for EH-domain and SH3 domain regulator of Endocytosis).

## Summary of the Invention

In accordance with one series of embodiments, this invention provides isolated nucleic acids corresponding to or relating to the nucleic acid sequences disclosed herein which encode the murine Ese1 and Ese2 proteins.

One of ordinary skill in the art is now able to identify and isolate mammalian Ese protein genes or cDNAs which are allelic variants of the disclosed sequences or are homologues thereof, in other species, including humans, using standard hybridisation screening or PCR techniques.

In a further embodiment, the invention provide cDNA sequences encoding murine Ese1 and Ese2 proteins comprising the nucleotide sequences of Sequence ID NOS:1, 2, 4 and 5.

Also provided are portions of the Ese gene sequences useful as probes or PCR primers or for encoding fragments, functional domains or antigenic determinants of Ese proteins.

The invention also provides portions of the disclosed nucleic acid sequences comprising about 10 consecutive nucleotides to nearly the complete disclosed nucleic acid sequences. The invention provides isolated nucleic acid sequences comprising sequences corresponding to at least 10, preferably 15 and more preferably at least 20 consecutive nucleotides of the Ese genes as disclosed or enabled herein or their complements.

In addition, the isolated nucleic acids of the invention include any of the above described nucleotide sequences included in a vector.

In accordance with a further series of embodiments, this invention provides substantially pure murine Ese proteins, fragments of these proteins and fusion proteins including these proteins and fragments.

In accordance with a further series of embodiments, this invention provides substantially pure mutant murine Ese proteins, fragments of these proteins and fusion proteins including these mutant proteins and fragments.

The proteins, fragments and fusion proteins have utility, as described herein, for the preparation of polyclonal and monoclonal antibodies to mammalian Ese proteins, for the identification of binding partners of the mammalian Ese proteins and for diagnostic and therapeutic methods, as described herein. For these uses, the present invention provides substantially pure proteins, polypeptides or derivatives of polypeptides which comprise portions of the mammalian Ese amino acid sequences disclosed or enabled herein and which may vary from about 4 to 5 amino acids to the complete amino acid sequence of the proteins. The invention provides substantially pure proteins or polypeptides comprising sequences corresponding to at least 5, preferably at least 10 and more preferably 50 or 100 consecutive amino acids of the mammalian Ese proteins disclosed or enabled herein.

The proteins of the invention may be isolated and purified by an conventional method suitable in relation to the properties revealed by the amino acid sequences of these proteins.

Alternatively, cell lines may be produced which express or over-express the Ese gene products, allowing purification of the proteins for biochemical characterisation, large-scale production, antibody production and patient therapy.

For protein expression, eukaryotic or prokaryotic expression systems may be generated in which an Ese gene sequence is introduced into a plasmid or other vector which is then introduced into living cells. Constructs in which the Ese cDNA sequence containing the entire open reading frame is inserted in the correct orientation into an expression plasmid may be used for protein expression. Alternatively, portions of the sequence may be inserted. Prokaryotic or eukaryotic expression systems allow various important functional domains of the proteins to be recovered as fusion proteins and used for binding, structural and functional studies and also for the generation of appropriate antibodies.

The present invention includes effective fragments, analogues of the Ese proteins described herein. "Effective" fragments or analogues retain the activity of the described Ese proteins to promote endocytosis via clathrin-coated pits. The term "analogue" extends to any functional and/or chemical equivalent of a mammalian Ese protein including mimetics and includes proteins having one or more conservative amino acid substitutions, proteins incorporation unnatural amino acids and proteins having modified side chains.

In accordance with a further embodiment of the invention, antibodies are enabled which bind specifically to the Ese proteins disclosed herein. Polyclonal or monoclonal

antibodies may be prepared using conventional methods. Antibodies may also be prepared to individual selected domains of the Ese proteins, as described herein.

In a further embodiment, the invention provides pharmaceutical compositions containing an Ese protein or a non-functional mutant Ese protein for the treatment of mammalian disorders which involve abnormal endocytosis leading to altered cellular functioning. Administration of a therapeutically active amount of a pharmaceutical composition of the present invention means an amount effective, at dosages and for periods of time necessary to achieve the desired result.

In accordance with a further embodiment, the invention provides a method for identifying binding partners of the Ese proteins disclosed herein. Such methods in general include various assays including those including radiolabelling of the Ese proteins. Other methods may include but are not restricted to phage display, affinity purification techniques, expression cloning and the yeast 2-hybrid system, as described herein.

In accordance with a further embodiment of the present invention, is a method for identifying proteins which phosphorylate Ese proteins. Such method includes known phosphorylation assays.

The identification of proteins or peptides interact with or bind to Ese proteins can provide the basis for the design of peptide antagonists or agonists of Ese protein function or for the design of peptide antagonists or agonists of Ese protein binding partners which affect Ese protein function. Further, the structure of these peptides determined by standard techniques such as protein NMR or x-ray crystallography can provide the structural basis for the design of improved small molecule drugs.

In accordance with a further embodiment, the present invention also provides for the production of mouse models or transgenic non-human animal models for the study of mammalian Ese gene function, for the screening of candidate pharmaceutical compounds, for the creation of *in vitro* mammalian cell cultures which express the Ese proteins or in which an Ese gene has been inactivated by knock-out deletion, and for the evaluation of potential therapeutic interventions.

The invention enables a transgenic animal, including a transgenic insect, wherein the genome of the animal or of an ancestor of the animal has been modified by introduction of a transgene comprising mammalian Ese genes under the transcriptional control of tissue restricted regulatory elements including the mouse mammary-tumour virus long term repeat sequences.

Transgenic animals with inappropriate expression of Ese proteins may be examined for phenotypic changes, for example abnormal cellular development or abnormal cellular signalling and may be used to screen for compounds with potential as pharmaceuticals. Compounds which provide reversal of the phenotypic changes are candidates for development as pharmaceuticals.

Transgenic animals in accordance with the invention can be created by introducing a DNA sequence encoding a selected Ese protein either into embryonic stem (ES) cells of a suitable animal such as a mouse, by transfection or microinjection, or into a germ line or stem cell by a standard technique of oocyte microinjection. Such methods of producing animal models are fully described in the literature.

In accordance with another aspect of the present invention is a method for screening a candidate compound for effectiveness as an antagonist of an Ese protein comprising:

(a) providing an assay method for determining the endocytotic regulatory capacity of an Ese protein; and

(b) determining the endocytotic regulatory capacity of the Ese protein in the presence or absence of the candidate compound, wherein a reduced level of endocytosis in the presence of the candidate compound indicates antagonist activity of the compound.

In accordance with another aspect of the present invention is a method for treating in a mammal a disorder associated with an undesired level of endocytotic activity of an Ese protein comprising administering to the mammal an effective amount of a substance selected from the group consisting of:

(a) an Ese protein antagonist;

(b) an antibody which binds specifically to an Ese protein;

(c) an antisense strand comprising a nucleic acid sequence complementary to the sequence or fragment of the sequence and capable of hybridizing to the nucleic acid sequence encoding an Ese protein;

(d) an agent which down regulates the expression of the Ese gene encoding for an Ese protein;

(e) an antagonist of an Ese protein binding partner; and

(f) an Ese agonist.

According to another aspect of the present invention is a method for suppressing in a mammal, the abnormal proliferation of a cell capable of being stimulated to proliferate by

a growth factor receptor, the method comprising administering to the mammal an effective amount of a Ese protein antagonist, an Ese agonist or an antibody which binds specifically to an Ese protein.

According to yet another aspect of the present invention is a method for preventing viral infection in a mammal, said method comprising administering to the mammal an effective amount of an Ese protein antagonist, an Ese agonist or an antibody which binds specifically to an Ese protein or an Ese mutant protein not capable of regulating endocytosis.

According to a further aspect of the present invention is a method for causing endocytosis in selected cells in a mammal in need of such treatment, said method comprising administering to the mammal an effective amount of an Ese protein or an active analogue, mimic or fragment thereof.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the invention are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

## Summary of Tables and Drawings

A detailed description of the preferred embodiments are provided herein below with reference to the following tables and drawings in which:

Table 1 shows alignment of mouse Ese1 and Ese2 protein sequences. EH, coiled-coil and SH3 domains are as indicated.

Figure 1 shows a Northern blot demonstrating expression of Ese1 and Ese2 genes in various adult tissues.

Figure 2 shows Western blots demonstrating the association of Ese and Eps15 proteins *in vivo*. Endogenous Ese1 proteins were precipitated with Rabbit anti-peptide antisera against the N-terminus of Ese1. Immunoprecipitates were then analyzed on western blots for the presence of Ese1/2 with Chicken anti-Ese antisera or for co-precipitation of Eps15 with Rabbit antisera raised against the C-terminus of Eps15.

Figure 3 shows association of Ese and Eps15 C-terminal deletion mutants. Cos-1 cells were transfected with pcDNA3Eps15, pcDNA3Ese1 or the C-terminal deletion mutants pcDNA3Ese1δC and pcDNA3Eps15δC-Flag as indicated. Cell lysates were

immunoprecipitated with rabbit anti-Ese1 or rabbit anti-Eps15 (left panel); rabbit anti-Ese1, mouse anti-Flag or rabbit anti-Eps15 (right panel). Panels represent western blots to detect the presence of Ese1 in each immunoprecipitation. A 90kDa Ese1 protein exists in the third and sixth lanes on the left panel which is the C-terminally truncated Ese1 protein which is co-immunoprecipitated in a complex with Eps15 in the sixth lane. Also, to be noted is the precipitation of Ese1 with anti-Flag monoclonal antibody in the sixth lane of the right panel experiment. In this case, Ese1 has been precipitated in a complex with the C-terminally truncated Eps15δC protein.

Figure 4 shows confocal immunofluorescent micrographs of Cos cells transfected with myc-Ese1 (A, A'); Eps15 (B); myc-Ese1 + Eps15 (C, C', C'',D) or myc-Ese1 + Eps15δC (E).

Figure 5 shows confocal immunofluorescent micrographs of Cos cells transfected with myc-Ese1 and probed with antibodies to myc (A), Dynamin (B) or both antibodies (C).

Figure 6 shows a proposed model for Ese regulation of Endocytosis.

In the drawings, preferred embodiments of the invention are illustrated by way of example. It is to be expressly understood that the description and drawings are only for the purpose of illustration and as an aid to understanding, and are not intended as a definition of the limits of the invention.

**Detailed Description of the Invention**

Sequencing of Murine Ese1 and Ese2 genes

The full length murine Ese1 cDNA was sequenced (Sequence ID NO:1). It encodes a sequence of 1213 amino acids (Sequence ID NO:3) having a predicted molecular weight of 137 kDa. Murine Ese2 cDNA was also sequenced (Sequence ID NO:4) and encodes a sequence of 1197 amino acids (Sequence ID NO:6) having a predicted molecular weight of 135.7 kDa..

The full length Ese proteins are predicted to encode two N-terminal EH domains followed by a coiled-coil domain and five SH3 domains (Table 1). Several Ese1 isolated clones contain only $SH3_A$, $SH3_B$ and $SH3_E$ domains. Indeed, the $SH3_C$ domain was not included in the original human SH3P17 partial cDNA. In addition, expressed sequence tags from the Ese1 gene have been found in the public databases which skip sequences encoding individual EH domains or regions of the coiled-coil domain indicating that this gene is subject to complex alternative splicing and has the potential to encode for many

distinct proteins. The predicted Ese2 protein on the other hand encodes a C-terminal extension of 45 amino acids in comparison to the human SH3P18 partial cDNA, suggesting that this gene is also alternatively spliced. The Ese proteins are 53% identical over the full length of Ese1 (645 of 1213 residues in Ese1 line up with identical residues in Ese2) and are related to the Ese protein from *Xenopus* which has recently been submitted to genebank (Accession # AF032118).

The two EH domains of Ese1 are highly related to the respective EH domains in Ese2 and these Ese EH domains are most closely related those found in Eps15 and Eps15R, two mammalian protein which are required for endocytosis through clathrin-coated pits. EH domains have also been identified in End3 and Pan1p which are yeast partners proteins required for endocytosis. The central third of both Ese proteins are predicted to encode an extended coiled-coil which is a domain typically associated with protein-protein association through dimerization or tetramerization as noted for Eps15 (29, 54). Besides the homology between Ese1 and Ese2, the Ese1 SH3 domains are most closely related to SH3 domains from Myosin IB in Acanthamoeba ($SH3_A$), Myosin IB in Entamoeba ($SH3_B$), the YFR024 hypothetic yeast protein ($SH3_C$), Myosin IB from Acanthamoeba ($SH3_D$) and Myosin IC from Acanthamoeba ($SH3_E$). The same homologies are noted for SH3 domains from Ese2 with the exception of $SH3_A$ which is most similar to the SH3 domain from βPIX, $SH3_C$ which is most similar to an SH3 domain from the mouse Ray protein and $SH3_D$ which is most similar to the SH3 domain from Dictyostelium myosin IB. Additional sequence analysis reveals the presence of a very large number of potential phosphorylation sites in the Ese proteins and a single SH3 binding consensus in the N-termini of each Ese protein.

Expression of Ese genes

In order to determine where the Ese genes are expressed we have performed Northern analysis on mRNA derived from several adult mouse tissues (Figure 1). These genes are both widely expressed and reveal a complex pattern of alternatively spliced transcripts. The highest levels of Ese1 mRNA were noted in brain, heart and skeletal muscle. Brain and skeletal muscle mRNA also contain an unusually large transcript which may be as much as 15kb in length. Interestingly, these tissues express high levels of many proteins involved in synaptic vesicle recycling and endocytosis. A prominent 2.4kb Ese2

transcript has also been noted which is significantly smaller than the 3591bp sequence required to code for the full Ese2 protein as shown in Table 1.

Subcellular localisation of Ese proteins

To determine the subcellular localization of Ese1, a myc-epitope tagged version of this protein (mycEse1) was engineered. This tagged protein was expressed in Cos cells and detected by confocal immunofluoresence using the mouse anti-myc monoclonal antibody 9E10 (Figure 4). Interestingly, the transfected Ese1 protein is highly concentrated into circular domains (Figure 4A and 4C) which are present throughout transfected cells (compare single optical section in Figure 4A with the projection of all sections through the same cell shown in 4A'). Ese1 overexpressed in 10T1/2, BHK and Hela cells using the vaccinia virus T7 expression system is also localized to large circular domains (data not shown). This concentration of ectopically expressed Ese1 contrasts with the localization of Eps15 in transiently transfected cells, Eps15 being dispersed throughout the cell (Figure 4B). Ese1 and Eps15 proteins form a complex *in vivo* and yet localize to distinct subcellular compartments in transfected Cos cells. We therefore determined the localization of both mycEse1 and Eps15 in Cos cells co-transfected with both genes. In co-transfected cells, mycEse1 is still found in circular domains (Figure 4C). Interestingly, the transfected Eps15 is now also partially co-localized with Ese1 in the same circular domains (Figure 4C' and C'').

The C-terminal third of Eps15 contains several regions which are required for association with a-adaptin of the AP2 clathrin adaptor complex (31). It has been shown that this region of Eps15 is not required for its association with Ese1 (Figure 4). In order to test whether Eps15 function may be required for Ese localization, mycEse1 and Eps15δC have been co-transfected. Interestingly, mycEse1 localization is completely altered in cells co-transfected with Eps15δC (compare Figure 4D and E) indicating that Ese1 requires Eps15 function for its distinctive subcellular localization. In addition, these data suggest that the Ese1:Eps15 complex must be associated with AP2 in order to form the large circular domains in Ese1 transfected cells.

The Dynamin GTPase is required for clathrin-coated vesicle fission and is recruited to coated pits by Amphiphysin I/II heterodimers (6). In order to study the subcellular localization of Dynamin in Ese1 overexpressing cells we have co-stained for the myc epitope tag with Rabbit anti-myc antisera and for endogenous Dynamin with mouse anti-Dynamin monoclonal antibodies. Surprisingly, endogenous Dynamin is recruited to the Ese1 staining circles and both proteins are co-localized (Figure 5). These results

demonstrate that mycEse1-containing circles are endocytic structures and that Ese1 is a rate limiting component of the early endocytic compartment which can function together with Eps15 to induce Dynamin recruitment.

Identification of binding partners of Ese proteins

In order to identify Ese partners, a systematic analysis was initiated of each domain for binding partners using the yeast two hybrid system. The central coiled-coil domain of Ese1 from amino acid 330 to 732 was fused to the GAL4 DNA binding domain and transformed into the Y190 reporter strain together with plasmid cDNA libraries from several tissue sources. Yeast colonies were selected for growth on Histidine minus plates to select for interaction between library encoded GAL4 activation domain fusions and the GAL4 DNA binding domain Ese1 coiled-coil bait. Yeast colonies which survived selection for expression of the His3 gene where also tested for induction of the integrated LacZ gene which is GAL4 responsive in Y190. Prey plasmids were recovered from 37 positive yeast colonies which were selected for expression of both His3 and LacZ. Several classes of cDNA were recovered in this screen. One class included Eps15 which was isolated twice and Eps15R which was isolated from four yeast colonies. Interestingly these clones were all partial cDNA fusions which minimally included C-terminal sequences from the central coiled-coil domain and downstream. In the case of Eps15, the positive clones start from amino acid 306 and 376 whereas in Eps15R the N-terminal boundary of clones were amino acid 4, 10, 222 and 386. These data indicate that minimal sequences required for interaction between the Ese1 coiled-coil domain and Eps15(R) include either or both of the central coiled-coil and/or the C-terminal third of these proteins.

The Ese proteins were analyzed *in vivo* to test for their association with Eps15 or Eps15R. Polyclonal antisera were raised in chickens against a GST fusion containing the C-terminus of Ese1 from amino acid 665 to the stop codon. This region of Ese1 contains all five of the SH3 domains. In addition, polyclonal antisera were generated in rabbits against a peptide representing the first 21 amino acids of Ese1. Cell lysates were prepared from A431, PC12, MDCK and Hela cells which represent cell lines from several distinct tissue types and species. The rabbit anti-peptide antisera were used to precipitate Ese1 from each lysate and precipitates were analyzed by western blotting using the chicken anti-Ese sera. In each cell line the presence of several specific bands in the range of 150kDa were observed which were precipitated in the absence but not in the presence of the peptide to which the sera was generated (Figure 2). Thus the Ese1 protein is expressed in

many tissue culture cells and runs in a range consistent with the predicted molecular weight of 137kDa. The same samples were also analyzed for co-immunoprecipitation of Eps15 proteins. In each case multiple Eps15 proteins were detected which co-purify with Ese1. The anti-Ese1 peptide antisera and commercially available anti-Eps15 antisera which were used were raised against epitopes which are not shared by these proteins indicating that Ese1 and Eps15 are constitutively associated partners *in vivo*. This association is reminiscent of the previously detected complex between two EH domain containing yeast proteins, End3p and Pan1p.

Ese1 and Eps15 proteins both contain central coiled-coil motifs. In addition, Ese1 contains multiple C-terminal SH3 domains while Eps15 contains SH3-binding motifs. In order to map the regions of each protein which are required for their association *in vivo*, C-terminal truncations of each (Eps15δC and Ese1δC) were generated. Full length Eps15 was co-transfected into Cos-1 cells together with either full length Ese1 or C-terminally truncated Ese1δC. Cell lysates were precipitated with either rabbit anti-Eps15 or with rabbit anti-Ese1, and precipitates were western blotted with chicken anti Ese1 antisera. Interestingly, the C-terminally truncated Ese1 protein was efficiently immunoprecipitated in a complex with Eps15 using anti-Eps15 sera. In a reciprocal experiment Cos-1 cells were transfected with Ese1 alone or together with Eps15 or C-terminally truncated Eps15 which had been Flag-epitope tagged (Eps15δC-Flag). Cell lysates were prepared and immunoprecipitated with either rabbit anti-Ese1, mouse anti-Flag, or rabbit anti-Eps15 antibodies. These immunoprecipitations were also western blotted to analyze for the presence of Ese1. The anti-Flag antibody efficiently precipitated Ese1 from cells expressing Flag-tagged Eps15δC indicating that the C-terminally truncated Eps15 protein can bind to Ese1 *in vivo*. The Ese1 and Eps15 proteins are therefore associated through interaction of their central coiled-coil regions and do not require the presence of SH3 and SH3-binding motifs in their respective C-termini.

In addition to the identification of Eps15 and Eps15R in the yeast two hybrid screen with the coiled-coil domain bait of Ese1, the following were also identified as Ese binding proteins: TSG101 (accession #U52945), meningioma expressed antigens 6/11 (accession #U94780 for mea6), β-tropomyosin, rabaptin5 (accession #D86066), Adora2a (accession #Y13345), L1 lipid binding protein (accession #K02109) and numerous cytokeratins and laminins. Novel genes identified in this screen are detailed below.

Also performed was a yeast two hybrid screen using amino acids 665-1213 of Ese1 as bait. This screen led to the isolation of the following clones which produced GAL4 Activation domain fusions which bound to this SH3 domain bait of Ese1. The Ese-binding proteins identified in our SH3 screen were the cbl-b oncoprotein (accession #U26712), Dynamin II (accession #L31398), KIAA0268 (accession #D87742), Jerky (accession #U35730), hnRNP-K (accession #L29769), SAP49 (accession #L35013). Novel genes identified in this screen are detailed below and several novel clones as outlined below.

These results demonstrate that the novel Ese genes and the proteins that they encode function in a complex with Eps15 proteins to regulate endocytosis together. In addition, this complex contains binding sites for numerous other proteins. Furthermore, with the identification of several potential phosphorylation sites on the Ese proteins, these results also suggest that whether or not complexed with Eps15, Ese proteins are involved in intracellular signalling processes which are likely to lead to altered cellular activity. Many Ese partners have been identified in these studies. Also revealed is a novel method to identify more Ese partners. Yeast cells have a complex formed by two EH domain proteins (Pan1p and End3p) which regulates both endocytosis as well as the actin cytoskeleton. Indeed, the Eps15 protein has been reported to regulate both endocytosis and the actin cytoskeleton (1). As Eps15 and Ese function together, and Ese contain many protein-protein interaction surfaces on this complex, strongly suggesting that the Ese proteins and their binding proteins are critical regulators of Eps15:Ese functions *in vivo*.

Taken together, these studies demonstrate the identification and isolation of two novel murine Ese proteins encoded by two novel murine Ese genes which are involved in the regulation of endocytosis via clathrin-coated pits. The process of endocytosis is most likely to be related to receptor-mediated endocytosis, however, may also be related to pinocytosis or non-receptor mediated endocytosis. The novel Ese proteins of the present invention appear to function to regulate endocytosis involving the formation of clathrin-coated pits by the polymerization of clathrin into a lattice along the cytosolic face of a region of membrane causing the region to expand inward. Ultimately, the pit pinches off from the membrane, and the clathrin cage is completed thus producing a coated vesicle. Through the binding of Esp15 to form an Ese-Eps15 complex via a central binding region, the complex can recruit other proteins such as assembly particles and dynamin to bind GTP and promote coated vesicle formation and perhaps their transport to appropriate locations within the cell and release of internalized proteins and/or molecules. The SH3 C-terminal domains of the Ese proteins can bind and interact with several other proteins

leading to a host of protein-protein interactions involved in the endocytotic process. The Ese proteins may be activated or inactivated via phosphorylation of the proteins at numerous phosphorylation sites by the action of activated receptors on cell surfaces.

Due to the fact that the Ese proteins appear to be a key central player in the complex process of endocytosis involving protein-protein interactions and intracellular signalling, these proteins are most likely involved in a myriad of clinical conditions and processes which are very likely to include but not be limited to regulation of endocytosis (as described above), cell division and cancer (Eps15 and cbl-b are oncoproteins), cell migration (regulation of the actin cytoskeleton is required for many forms of cell migration), cell polarity, plane of cell division and cell fate specification (Eps15 binds to Numb *in vivo* which is required for these processes(43), RNA localization (several RNA binding proteins have been identified in the present screens) and viral infection and life cycle (Eps15 binds to RAB a cellular cofactor for HIV Rev (43)).

With respect to viral infection Ese proteins may play an important role and thus may be a target for developing therapeutic strategies against viral infection and virally-induced disease states. HIV is known to alter endocytosis of several important cell surface molecules including CD4 and MHC antigens. HIV has been shown to bind to NEF which has been demonstrated to bind to SH3 sites. This binding to NEF induces clathrin coated pit formation. As NEF binds SH3 sites and induces endocytosis, it is possible that Ese proteins may bind to NEF and are involved in the endocytotic process. Therapeutic strategies to provide treatment for viral infection and virally induced disease states may therefore include the inhibition of Ese-NEF binding using antagonists, antibodies or other agents directed against such a complex to inhibit endocytosis and in this manner inhibit viral infection and virally induced disease states. It is also expected that many types of viruses will interact with the multi-component Eps15-Ese complex.

Synaptic transmission and abnormal or altered synaptic transmission as seen in various nervous disorders may also be a target for the therapeutic use of Ese proteins and/or antagonists. The Eps15:Ese complex has been demonstrated to regulate endocytosis, is highly expressed in the brain and Ese binds to Jerky; a protein required to prevent epilepsy in mice (57). Futhermore, Ese proteins are highly expressed not only in the brain but also in the heart and in skeletal muscle which are tissues involving high levels of synaptic transmission. This suggests that Ese protein may be used in the treatment of nervous system disorders involving altered synaptic transmission.

Receptor-mediated cell signaling such as seen with several different types of growth factors also involves endocytosis. Over-expression, mutation or over-stimulation of growth factor receptors has been demonstrated to lead to abnormal cell division and growth as seen in cancer. For example, EGF is a potent mitogen for many epithelial cells and EGF receptor activation is known to stimulate intracellular kinase pathways leading to cell proliferation. Such activity may play a role in cancer progression.By altering the rate of endocytosis by targeting Ese proteins, the cell proliferative effect of growth factor receptor stimulation may be counteracted.

Abnormal cell division and cell migration is seen in several diseases and involves the cell cytoskeleton. The intracellular cytoskeleton is highly organized and consists of microtubules, microfilaments and intermediate filaments acting as an internal reinforcement in the cytoplasm of a cell. Together these structures associate in a regular and defined manner which is regulated by extracellular signals and may transduce plasma membrane signals by association with other proteins or by second messengers. The Ese-Eps15 complex is very likely to regulate the cytoskeleton by analogy to the role of Pan1p:End3p in regulation of yeast cytoskeleton. Furthermore, endocytosis is known to involve a rearrangement of the intracellular cytoskeleton. Cell division and migration require the continual rearrangement of the intracellular cytoskeleton. Therefore, abnormal patterns of cell division and migration may involve altered Ese function and altered endocytosis. The Ese protein or the gene may therefore be used to alter regulation of endocytosis or the association of the Ese protein with the cell cytoskeleton to restore cell division and migration to normal levels and patterns.

Tissue development also involves the continual remodeling of the extracellular and intracellular cytoskeletal network along with associated proteins. Developmental diseases can occur as a result of abnormal remodeling of the cytoskeleton leading to altered intracellular signaling. As Ese proteins are likely to be involved with both the cytoskeleton and intracellular signaling they may also be directly involved in the development of certain developmental diseases and therefore may be a target for therapeutic treatment of such diseases. Ese proteins may also be involved in normal development including that of stem cells which are self-renewing cells that divide to produce differentiated daughter cells in various tissues. As Ese proteins are associated with the cytoskeleton they may play a part in the formation of certain types of differentiated cells through the partitioning of RNA and proteins such as NuMb during cell division.

To summarize, Ese proteins, Ese complexes including Eps15, Eps15R and many of the proteins identified in the screens as well as others identified through similar screens can be targeted for use in therapies to treat diseases including cancer, viral infection based diseases, developmental diseases due to altered cell fate specification and/or division as well as neurological diseases and diseases of altered cell migration and other diseases due to defects in the actin cytoskeleton.

## EXAMPLES

The examples are described for the purposes of illustration and are not intended to limit the scope of the invention.

Methods of molecular genetics, protein and peptide biochemistry and immunology referred to but not explicitly described in this disclosure and examples are reported in the scientific literature and are well known to those skilled in the art.

Material and Methods

Ese cloning and plasmids

High Stringency screening was used to isolate the two mouse Ese cDNAs by previously described methodology (58). Ese1 was cloned from an adult mouse brain cDNA library using a PCR product from nt 1707-2197 of the coding sequence as probe. Ese2 was cloned from a mixed tissue adult mouse cDNA library using a mixture of three probes (EST#583881 (Research Genetics Inc.), EST#652549 (Research Genetics Inc.) and a PCR product from nt 2712 to nt 3456 of the Ese2 coding sequence. The mouse Eps15 cDNA was generated through a combination of high stringency library screening with Est sequences from the Eps15 gene and rt PCR according to established methods.

pcDNA3Ese1:

Full length Ese1 was cloned into the Not1 site of pcDNA3 (Invitrogen Inc.). The Ese1 cDNA includes 53 nucleotides of 5' UTR plus a natural NotI site and 288 nucleotides of 3'UTR plus a small region of polylinker including a NotI site.

pcDNA3mycEse1:

The 5' end of pcDNA3Ese1 from the EcoRI site in the pcDNA3 polylinker to the start codon was replaced with the DNA sequence

GAATTCAGAACCATGGAACAAAAGCTTATTTCTGAAGAAGACTTGGGGCCCATG:

where the first underline corresponds to an EcoRI site which was fused into the pcDNA3 EcoRI site and the extended underlined sequence codes for a myc-epitope tag. This is followed by nine nucleotides which code for glycine, proline and the natural Ese1 start codon. This sequence was joined to the sequences coding for amino acids 2-1213 (the remainder of Ese1). The new start codon in this tagged Ese1 construct is bolded. The 3' end of Ese1 in this vector is the same as in pcDNA3Ese1 above.

pcDNA3Ese1δC:

The C-terminus of Ese1 was removed by replacing all sequences from nt 2209 of the coding sequence to the XhoI site in pcDNA3Ese1 with **TGA**CTCGAG where the stop codon is in bold and the XhoI site is underlined. This construct codes for amino acids 1-736 of Ese1.

pCDNA3Eps15:

This plasmid was constructed from four pieces. It contains the full length Eps15. The 5'UTR of this construct has been constructed to be GGATCCACC**ATG** where a BamHI site is underlined and the start codon is bolded. This BamHI site was fused to the BamHI site in pcDNA3. The 3'UTR in this vector is 204 nt of the mouse natural 3'UTR fused to a short cloning linker ending in the sequence AAGCTTGGGCCC where an ApaI site is underlined; this ApaI site was fused to the ApaI site in pcDNA3.

pcDNA3Eps15δC:

This vector is the same as pcDNA3Eps15 except that sequences downstream from and including mouse Eps15 coding nucleotide 1500 have been replaced with CCTGGATTACAAGGATGATGATGACAAA**TGA**CTCGAG where the first underlined sequence codes for the Flag-epitope, an inframe stop codon is bolded and an XhoI site is underlined. This XhoI site was fused to the polylinker in pcDNA3. The resulting plasmid encodes amino acids 1-501 of mouse Eps15 fused to a C-terminal Flag epitope. The 5' end of Eps15 in this construct is as indicated above for pcDNA3Eps15.

pGBT9Ese1cc:

The Ese1 sequence coding for amino acids 330 to 732 were fused directly to GAATTC (EcoRI site) on the 5' end and to **TA**GGATCC (stop codon followed by a

BamHI site) on the 3' end. This fragment was cloned into EcoRI/BamHI digested pGBT9 in frame with the GAL4 DBD.

pGBT9Ese1A3.3:

This plasmid encodes the bait for our SH3 screen. It encodes all five SH3 domains from amino acid 665-1213 and was subcloned into pGBT9 on an EcoRI fragment which fuses the Ese1 SH3 region in frame with the DBD of GAL4.

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Yeast 2-Hybrid Screening

pGBT9Ese1cc was transformed into *S. cerevisiae* strain Y190 using 45% PEG4000, 100 mM LiAc, 10 mM Tris-HCl (pH 7.5) as per standard protocols. Cells were plated onto Sc-Trp drop-out media. Single colonies were isolated and expression of bait was analyzed by Western Blot using antibodies against the GAL4 DBD. A clone expressing the bait fusion was used to inoculate a 100 mL Sc-Trp liquid culture grown overnight at 30°C. Cells were then re-inoculated into YPD at a density of $5x10^6$ cells/mL and grown at 30°C until the titer reached $2x10^7$ cells/mL. Cells were pelleted, resuspended in 50 mL 100 mM LiAc and incubated for 10 min at 30°C. Once again the cells were pelleted, resuspended in 20 mL PLA [35% PEG, 100 mM LiAc, 2 mg/mL salmon sperm DNA] containing 30 µg plasmid library (cloned in pAD-GAL4) incubated at 30°C for 30 min.; then heat shocked at 42°C for 40 min., pelleted, resuspended in water and plated onto [Sc-Trp-Leu-His+40 mM 3-AT]. Plates were incubated at 30°C until colonies were formed. Colonies were picked, patched and grown at 30°C overnight on Whatman filter paper laid on top of Sc-Trp-Leu-His plates. Filters were submerged in liquid nitrogen for 15 seconds then placed on top of blotting paper soaked in Z-buffer + X-gal. β-galactosidase activity was measured by the appearance of blue colour. Plasmids from β-galactosidase positive colonies were shuttled to bacteria by electroporation and isolated for sequencing.

Northern Blot analysis

A multiple tissue northern blot (Clontech) was prehybridized in 5 mL of ExpressHyb Solution (Clontech) at 68°C for 30 min. Probe was added at $1x10^6$ cpm/mL for 1 hr. The Blot was washed twice [2X SSC, 0.05% SDS] at room temperature, twice [0.1X SSC, 0.1% SDS] at 50°C and then exposed to film overnight.

Antibodies and Western Blot analysis

Western blot analysis was performed according to standard protocols. Briefly, cultured cell lines or 48 hours post tranfection Cos-1 cells where washed with PBS and lysed in one ml of cold lysis buffer (50 mM Hepes pH 7.4, 150 mM NaCl, 10% glycerol, 1% Triton X100, 1 mM EGTA, 1.5 mM $MgCl_2$, 10 mM NaF, 10 mg $ml^{-1}$ aprotinin, 1 mM PMSF, 10 mg $ml^{-1}$ leupeptin, 1 mM $Na_3VO_4$). Supernatants were clarified by centrifugation and immunoprecipitated with specific antiserum as indicated. Antigen-Antibody complexes where purified on anti-rabbit agarose or anti-mouse agarose (Sigma Chemical Co.) Samples were run on 7.5% PAGE gels and transferred to nitrocellulose membranes. Filters were blocked in 5% dry milk powder/0.05% Tween 20/PBS, washed in 1% dry milk powder/0.05% Tween 20/PBS, and probed with the appropriate antisera at 1mg/ml in wash buffer (In the case of chicken anti-Ese1 we used 10μg/ml to probe western blots). Probed filters were further washed, probed again with 1/5000 dilution of horseradish peroxidase conjugated anti-rabbit IgG, anti-mouse IgG antibodies (Amersham), or anti-chicken-IgY (Zymed); washed and signal detected using the Enhanced Chemiluminescence detection system as per manufacturers instructions (Amersham).

Rabbit anti-Ese1 antisera was raised against a peptide of the following sequence: MAQFPTPFGGSLDVWAITVEE. The antisera was affinity purified over the same peptide (Research Genetics). This peptide was also used at 5μg to compete for the 5μg of antibody per immunoprecipitation reaction. Chicken anti-Ese1 antisera was raised against a fusion protein between GST and amino acids 665-1213 of mouse Ese1. This sera was cleared of antibodies reacting against GST by incubation with glutathione s-transferase on glutathione agarose beads.

Immunofluorescence

For Immunofluorescent staining, Cos-1 cells were typically plated at a density of $2x10^5$ per 22x50mm coverglass and transfected with 2.5μg of plasmid using Superfect (Qiagen Inc.). After 2 hours, the cells were washed with 10%FBS in Iscove's Media and fed with fresh 10%FBS in Iscove's Media. Two days later, these cultures where fixed with cold methanol for 30 minutes at room temperature. Cultures were washed three times 10 minutes with Phosphate buffered saline (PBS), blocked for one hour at room

temperature with 1%BSA in PBS and then incubated with primary antibody in blocking solution for one hour, also at room temperature. Slides were then washed three times 10 minutes with PBS, incubated with secondary antibody/1% BSA/PBS in the dark for one hour at room temperature. Finally, slides were washed three times 10 minutes in the dark at room temperature and mounted using Dabco anti-fade solution (Sigma Chemical Co.). Slides were analyzed on the confocal microscope using the 63x objective and optical filters to separate signals on each channel.

For primary antibodies Mouse anti-myc monoclonal 9E10 (10μg/ml: Santa Cruz Biotech.), Rabbit anti-myc (5μg/ml: Upstate Biotechnology Inc.), Rabbit anti-Eps15 antisera #C20 (1μg/ml: Santa Cruz Biotech), Rabbit anti-Flag epitope antisera (5μg/ml: Zymed) and mouse anti-Dynamin I #D25520, which recognizes Dynamin in Cos-1 cells by both immunoprecipitation and western blotting (data not shown) (20μg/ml: Transduction labs, Inc.) were used. As secondary antibodies we used FITC-labelled goat anti-mouse (1:80 dilution) and Texas Red-labelled goat anti-rabbit antibodies (1:100 dilution) (Jackson Immuno Research Laboratories Inc.).

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Mouse Ese1 cDNA - Sequence ID NO:1

CGGCACGAGGAGGAGTGGAGCGGCGCGGGAGGGCGCGCAGCTTGGTTGCTCC
GTAGTACGGCGGCTCGCAAGGGAGCATCCCGAGCGGGCTCCGGGACGGCCGG
GAGGCAGGCAGGCGGGCGGGCGGGGATGGTGTGCGCGGCTGCGGACTCGGCG
TTCCTCGCGCGGCGTGCGGGCTGCACTGATTTGTGTGAGGGGCGGCCGCGCGC
ACCCGCCCGGAGATGAGGCGTCGATCAGCAAGGTGAACGTAATAGAACCATG
GCTCAGTTTCCCACACCTTTCGGTGGTAGCCTGGATGTCTGGGCCATAACTGT
GGAGGAAAGGGCCAAGCATGACCAGCAGTTCCTTAGCCTGAAGCCGATAGCG
GGATTTATTACTGGTGATCAAGCGAGGAACTTTTTTTTCCAATCTGGGTTACCT
CAGCCTGTCTTAGCACAAATATGGGCGCTAGCGGACATGAATAACGATGGAA
GGATGGATCAAGTGGAATTTTCCATAGCCATGAAGCTTATCAAACTGAAGCTA
CAAGGATATCAGCTCCCCTCCACACTTCCCCCTGTCATGAAACAGCAACCAGT
GGCTATTTCCAGTGCACCAGCATTTGGTATAGGAGGGATTGCTAGCATGCCAC
CACTCACAGCTGTTGCTCCTGTGCCAATGGGCTCCATTCCAGTTGTTGGAATGT
CTCCACCCTTAGTATCTTCTGTCCCTCCAGCAGCAGTGCCTCCCCTGGCTAACG
GGGCTCCTCCCGTCATACAGCCTCTGCCTGCGTTTGCGCATCCTGCAGCCACAT
GGCCAAAGAGTTCTTCCTTCAGCAGATCTGGTCCAGGGTCACAATTAAACACT
AAGTTACAGAAGGCACAATCATTCGATGTCGCCAGCGCCCCTCCAGCAGCAGA
ATGGGCTGTGCCTCAGTCATCAAGGCTGAAATACAGGCAGTTATTCAACAGCC
ACGACAAAACTATGAGTGGACACTTAACAGGTCCCCAGGCAAGAACTATTCTC
ATGCAATCAAGTTTACCCCAGGCTCAGCTGGCTTCAATATGGAATCTTTCTGA
CATTGATCAAGATGGAAAACTCACTGCAGAAGAATTTATCCTAGCTATGCACC
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AGCAACTCCAGAAGCAGAGGTCCCTGGAGGCAGCGCGACTGAAGCAGAAAGA
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Mouse Ese1 coding sequence - Sequence ID NO:2

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ACCTCAGCCTGTCTTAGCACAAATATGGGCGCTAGCGGACATGAATAACGATG
GAAGGATGGATCAAGTGGAATTTTCCATAGCCATGAAGCTTATCAAACTGAAG
CTACAAGGATATCAGCTCCCCTCCACACTTCCCCCTGTCATGAAACAGCAACC
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GATTACACCGCCCAGAACGATGACGAACTAGCCTTCAGCAAAGGCCAGATCAT
CAACGTCCTCAACAAGGAGGACCCGGACTGGTGGAAAGGAGAAGTCAGTGGG
CAAGTTGGGCTCTTCCCATCCAATTATGTAAAGCTGACCACAGACATGGACCC
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CAAAGAGACCCACTATCCCATATCACTGCCCAGAGGGATGA

Mouse Ese1 protein - Sequence ID NO:3

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CRLATQRQEIESTNKSRELRIAEITHLQQQLQESQQMLGRLIPEKQILSDQLKQVQ
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PGWLGGELKGKTGWFPANYAEKIPENEVPTPAKPVTDLTSAPAPKLALRETPAPL
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AIPGEEFIAMYTYESSEQGDLTFQQGDVIVVTKKDGDWWTGTVGDKSGVFPSNY
VRLKDSEGSGTAGKTGSLGKKPEIAQVIASYAATGPEQLTLAPGQLILIRKKNPGG
WWEGELQARGKKRQIGWFPANYVKLLSPGTSKITPTE
LPKTAVQPAVCQVIGMYDYTAQNDDELAFSKGQIINVLNKEDPDWWKGEVSGQ
VGLFPSNYVKLTTDMDPSQQ

Mouse Ese2 cDNA - Sequence ID NO:4

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CAGCCGTGACAGGCTGCGCAACAGGTTCGCTGCGGCCGGCCTGACGACTGAC
CCGGCGGCGGCGGCCGCGGCACGGCAGGGTCTTCCCGGAGCTTGGCCGCGCC
CACGCGCCGGTGTCGAGGAGCGCGCGGGGTCGCGCCGGGACGTGCGCGAGGC
GCCAGATGGCTGAGAGCTGCAAGAAGAAGTCAGGATCATGATGGCTCAGTTT
CCCACAGCGATGAATGGAGGGCCAAATATGTGGGCTATTACATCTGAAGAAC
GTACTAAGCATGATAAACAGTTTGATAACCTCAAACCTTCAGGAGGTTACATA
ACAGGTGATCAAGCCCGTACTTTTTTCCTACAGTCAGGTCTGCCGGCCCCGGT
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AGTTTATGAATGTAAAAAAAAAAAAAAAAAAAA

Mouse Ese2 coding sequence - Sequence ID NO:5

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TCTGTTAGTACATCCTCATTACCAAATGGAACTGCCAGTCTCATTCAGCCTTTA
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CCTGGAGCAGCAGGAAAACTGGTGGTTTGGGGAGGTGCACGGAGGAAGAGGA
TGGTTCCCCAAGTCTTATGTCAAGCTCATTCCTGGGAATGAAGTACAGCGAGG
AGAGCCAGAAGCTTTGTATGCAGCTGTGACTAAGAAACCTACCTCCACAGCCT
ATCCAGTTACCTCCACAGCCTATCCAGTTGGAGAAGACTACATTGCACTTTATT
CATACTCAAGTGTAGAGCCCGGGGATTTGACTTTCACTGAAGGTGAAGAAATT
CTAGTGACCCAGAAAGATGGAGAGTGGTGGACAGGAAGTATTGGAGAGAGAA
CTGGAATCTTCCCGTCCAACTACGTCAGACCAAAGGATCAAGAGAATTTTGGG
AATGCTAGCAAATCTGGAGCATCAAACAAAAAACCCGAGATCGCTCAAGTAAC
TTCAGCATATGCTGCTTCAGGGACTGAGCAGCTCAGCCTTGCGCCAGGACAGT
TAATATTAATCTTAAAGAAAAACACAAGCGGGTGGTGGCAAGGAGAGCTACA
GGCCAGAGGGAAGAAACGACAGAAGGGATGGTTTCCTGCCAGCCATGTAAAG
CTGCTAGGTCCAAGCAGTGAAAGAACCATGCCTACTTTTCACGCTGTATGTCA
AGTGATTGCTATGTATGACTACATGGCGAATAACGAAGATGAGCTCAATTTCT
CCAAAGGACAGCTGATTAATGTTATGAACAAAGATGACCCTGACTGGTGGCAA
GGAGAAACCAATGGTCTGACTGGTCTCTTTCCTTCAAACTATGTTAAGATGAC
AACAGACTCAGATCCAAGTCAACAGTGGTGTGCTGACCTCCAAGCCCTGGACA
CAATGCAGCCTACGGAGAGGAAGCGACAGGGCTACATTCACGAGCTCATTCA
GACAGAGGAGCGGTACATGGACGACCTGCAACTTTTTGAACAAAAAACTCTCC
TTTGA

mouse Ese2 - Sequence ID NO:6

MAQFPTAMNGGPNMWAITSEERTKHDKQFDNLKPSGGYITGDQARTFFLQSGLP
APVLAEIWALSDLNKDGKMDQQEFSIAMKLIKLKLQGQQLPVVLPPIMKQPPMFS
PLISARFGMGSMPNLSIHQPLPPVAPIATPLSSATSGTSIPPLMMPAPLVPSVSTSSL
PNGTASLIQPLSIPYSSSTLPHASSYSLMMGGFGGASIQKAQSLIDLGSSSSTSSTAS
LSGNSPKTGTSEWAVPQPSRLKYRQKFNSLDKGMSGYLSGFQARNALLQSNLSQT
QLATIWTLADIDGDGQLKAEEFILAMHLTDMAKAGQPLPLTLPPELVPPSFRGGK
QVDSVNGTLPSYQKTQEEEPQKKLPVTFEDKRKANYERGNMELEKRRQVLMEQ
QQREAERKAQKEKEEWERKQRELQEQEWKKQLELEKRLEKQRELERQREEERR
KEIERREAAKQELERQRRLEWERLRRQELLSQKTREQEDIVRLSSRKKSLHLELEA
VNGKHQQISGRLQDVQIRKQTQKTELEVLDKQCDLEIMEIKQLQQELKEYQNKLI
YLVPEKQLLNERIKNMQLSNTPDSGISLLHKKSSEKEELCQRLKEQLDALEKETAS
KLSEMDSFNNQLKELRESYNTQQLALEQLHKIKRDKLKEIERKRLEQIQKKKLED
EAARKAKQGKENLWRESIRKEEEEKQKRLQEEKSQDKTQEEERKAEAKQSETAS
ALVNYRALYPFEARNHDEMSFSSGDIIQVDEKTVGEPGWLYGSFQGKFGWFPCN
YVEKVLSSEKALSPKKALLPPTVSLSATSTSSQPPASVTDYHNVSFSNLTVNTTWQ
QKSAFTRTVSPGSVSPIHGQGQAVENLKAQALCSWTAKKENHLNFSKHDVITVLE
QQENWWFGEVHGGRGWFPKSYVKLIPGNEVQRGEPEALYAAVTKKPTSTAYPV
TSTAYPVGEDYIALYSYSSVEPGDLTFTEGEEILVTQKDGEWWTGSIGERTGIFPS
NYVRPKDQENFGNASKSGASNKKPEIAQVTSAYAASGTEQLSLAPGQLILILKKNT
SGWWQGELQARGKKRQKGWFPASHVKLLGPSSERTMPTFHAVCQVIAMYDYM
ANNEDELNFSKGQLINVMNKDDPDWWQGETNGLTGLFPSNYVKMTTDSDPSQQ
WCADLQALDTMQPTERKRQGYIHELIQTEERYMDDLQLFEQKTLL

Mouse Ese2 alternative transcript partial cDNA sequence - Sequence ID NO:7

CCGTCTTCCACATTTCCCACATTGATCGTGTGTACACACTCCGAACAGACAAC
ATCAACGAGAGGACGGCCTGGGTCCAGAAGATCAAGGGTGCCTCAGAGCAGT
ACATCGACACTGAGAAGAAGAAACGGGAAAAGGCTTACCAAGCCCGTTCTCA
AAAGACTTCAGGTATTGGGCGTCTGATGGTGCATGTCATTGAAGCTACAGAAT
TAAAAGCCTGCAAACCAAACGGGAAAAGTAATCCATACTGTGAAGTCAGCAT
GGGCTCCCAAAGCTATACCACCAGGACCCTGCAGGACACACTAAACCCCAAGT

GGAACTTCAACTGCCAGTTCTTCATCAAGGATCTTTACCAGGACGTTCTGTGTC
TCACTATGTTTGACAGAGACCAGTTTTCTCCAGATGACTTCTTGGGTCGTACTG
AAGTTCCAGTGGCAAAAATCCGAACAGAACAGGAAAGCAAAGGCCCCACCAC
CCGCCGACTACTACTGCACGAAGTCCCCACTGGAGAAGTCTGGGTCCGCTTTG
ACCTGCAACTTTTTGAACAAAAAACTCTCCTTTGAGGGCCTGGGGAAGCCAGA
ACCAGGGGAGCTGCCCACAAGGCTGGGTCTAAAGACAGATTTTGCTCTCCCAG
GACAGAGGAGCATCACATGGCTTCATCCATCAAACAGCCACACTCGCTGGGCC
TGTATTTTATTGCACACTAAATTGCTAGCAATCTATGCAAACATGATCTTT

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Mouse Ese2 alternative partial protein containing C2 membrane-binding domains - Sequence ID NO:8

VFHISHIDRVYTLRTDNINERTAWVQKIKGASEQYIDTEKKKREKAYQARSQKTS
GIGRLMVHVIEATELKACKPNGKSNPYCEVSMGSQSYTTRTLQDTLNPKWNFNC
QFFIKDLYQDVLCLTMFDRDQFSPDDFLGRTEVPVAKIRTEQESKGPTTRRLLLHE
VPTGEVWVRFDLQLFEQKTLL

**Novel Ese-coiled-coil interacting clones:**

Mouse homologue of C07E3.1 protein (clone 65): - Sequence ID NO:9

GAATTCGGCACGAGGGCTGAGAGAAGCGGACTCCGAGGACTCTGATGCTGAA
GAGAAGCCTGTTAAGCAGGAGGACTTCCCGAAGATTTAGGACCAAAGAAGTT
AAAGACGGGTGGCAATTTTAAGCCCAGCCAGAAAGGCTTTTCAGGAGGAACC
AAGTCCTTCATGGACTTTGGCAGCTGGGAGAGACACACGAAAGGGATCGGGC
AGAAGCTGCTGCAGAAGATGGGCTACGTCCCTGGGCGTGGCCTGGGGAAGAA
CGCACAGGGGATCATCAACCCCATCGAAGCCAAACAGAGAAAAGGCAAGGGA
GCCGTGGGGGCCTATGGCTCGGAGAGGACCACTCAGTCTCTGCAGGACTTCCC
CGTGGCCGACTCGGAAGAGGAGGCAGAAGAGGAGTTTCAGAAGGAGCTGAGC
CAATGGAGGAAAGACCCCAGCGGGAGCAAGAAGAAGCCAAAGTACTCTTACA
AGACTGTGGAGGAGCTGAAGGCCAAGGGCAGGGTCAGCAAGAAGCTCACAGC
ACCTCAGAAGGAACTGTCTCAGGTCAAGGTGATCGACATGACAGGCCGGGAG
CAGAAGGTGTACTACAGCTACAGCCAAATCAGCCACAAGCACAGCGTGCCCG
ATGAAGGGGTGCCATTGCTGGCGCAGCTGCCCCCCACAGCCGGCAAGGAAGC
CAGGATGCCGGGCTTTGCACTGCCTGAGCTGGAGCACAACCTGCAGCTGCTCA

TTGAGCGCACGGAGCAGGAGATCATCCAGAGCGACCGGCAGCTCCAGTATGA
GCGGGACATGGTGGTCAGCCTGTCGCATGAGCTGGAGAAGACGGCCGAGGTT
CTTGCACATGAGGAGCGTGTCATCTCTAACCTCAGCAAGGTGCTGGCCCTGGT
GGAGGAATGTGAGCGCCGCATGCAGCCCCATGGCACCGACCCCCTCACTCTGG
ATGAGTGTGCCCGCATCTTTGAGACACTACAGGACAAGTATTATGAGGAGTAC
CGCCTGGCGGACCGCGCAGACCTCGCTGTGGCCATTGTCTACCCGCTCGTGAA
GGACTACTTTAAGGATTGGCACCCCTCGAGGG

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Mouse partial C07E3.1 protein (clone 65): - Sequence ID NO:10

GTKSFMDFGSWERHTKGIGQKLLQKMGYVPGRGLGKNAQGIINPIEAKQRKGKG
AVGAYGSERTTQSLQDFPVADSEEEAEEEFQKELSQWRKDPSGSKKKPKYSYKT
VEELKAKGRVSKKLTAPQKELSQVKVIDMTGREQKVYYSYSQISHKHSVPDEGV
PLLAQLPPTAGKEARMPGFALPELEHNLQLLIERTEQEIIQSDRQLQYERDMVVSL
SHELEKTAEVLAHEERVISNLSKVLALVEECERRMQPHGTDPLTLDECARIFETLQ
DKYYEEYRLADRADLAVAIVYPLVKDYFKDWHPSR

Mouse Novel cDNA clone 42/ Est accession #W29719 and #AA915044: - Sequence ID NO:11

CATGGCGGCGGCTGCGGAGGGCGTCCCGGCGACGCGACGGAGGACGAGCCAC
CTCGAGATGATGCTGCGGTGGAGACAGCCGAGGAAGCAAAGGAGC

Mouse Novel cDNA clone 70: - Sequence ID NO:12

CTTGAGTCTACTGAAAATACCCTGCAGGAAGCTACATCATCCATGTCTTTGAT
GACCCAATTTGAACAGGAAGTATCTGGCCTCCAAAGACCATACGTGATATTGA
GACTAGCGAAGAGATGC

Mouse Novel cDNA clone 83/Est accession #AA589041 and W98708: - Sequence ID NO:13

GAATTCGGCACGAGGGAGTCTGGTTCTGGAAAGCCGACAGAAGCTGAGCTTG
TCAACTTAGATTTCTTGGGAGATTTGGATGTTCCGGTATCTGCCCCACCCCTGT
GTGTCTGAGCTCGAGTCTCTCTGCTGGACTATGG

**Novel Ese-SH3 interacting clones:**

Mouse YNK1 partial cDNA: - Sequence ID NO:14

CTTTACGAGCAGAGGGAGCCAAATTCAGAGCCGTTTTAGATAAAGCTGTGCAA
GCGGATGGACAGGTGAAGGAGCGCTACCAGTCCCATCGAGACACCATCGCAC
TTCTGTGTAAGCCGGAGCCAGAGCTGAATGCTGCCATCCCCTCTGCTAACCCA
GCAAAGACCATGCAGGGCAGCGAGGTTGTAAGTGTCTTAAAGTCCTTATTATC
AAATCTTGATGAAATCAAGAAGGAAAGAGAGAGTCTTGAGAATGACCTGAAG
TCAGTGAATTTTGACATGACAAGCAAGTTTTTGACAGCTCTGGCCCAAGATGG
CGTGATAAATGAGGAGGCTCTCTCTGTCACTGAGCTGGATCGGATCTATGGCG
GTCTAACAAGTAAAGTTCAAGAGTCTCTGAAGAAACAAGAGGGACTTCTAAAA
AATATACAGGTCTCACACCAAGAATTCTCCAAAATGAAGCAATCTAACAACGA
GGCTAACTTGAGAGAAGAAGTTCTGAAGAACCTAGCAACTGCGTATGACAACT
TTGTTGAGCTTGTAGCTAACTTGAAGGAGGGCACAAAGTTTTACAATGAGCTG
ACTGAGATCCTGGTCAGGTTCCAGAACAAATGCAGTGACATAGTGTTTGCACG
GAAGACAGAAAGAGACGAGCTCTTGAAGGATCTGCAGCAGAGCATTGCCAGA
GAGCCCAGCGCTCCTTCAATCCCTCCTCCAGCCTATCAGTCCTCCCCAGCAGC
GGGGCATGCAGCAGCGCCTCCAACTCCAGCCCCAAGAACCATGCCGCCTGCTA
AGCCCCAGCCTCCAGCCCGGCCTCCACCTCCTGTGCTTCCTGCAAACCGAGTT
CCTCCTGCTTCTGCTGCTGCTGCCCCTGCAGGCGTGGGGACGGCTTCAGCAGC
GCCGCCACAGACCCCTGGCTCTGCTCCCCCGCCACAGGCTCAGGGACCACCAT
ACCCTACCTATCCAGGATATCCCGGGTATTGCCAAATGCCCATGCCCATGGGC
TACAACCCCTACGCATATGGCCAGTACAATATGCCGTACCCACCGGTGTATCA
CCAGAGCCCCGGACAGGCTCCATACCCAGGACCCCAGCAGCCTACCTACCCCT
TCCCTCAGCCCCCGCAGCAGTCCTACTATCCACAGCAGTAACGCTGCCACGTG
CTGCTGGTTCAGATCAGAGCGACAGGACAGCAGCTGCCACCAGCTCTAAGCCA
CGCTCTGGCCACTCGAGAGTATCTTGCTCTATTGATTGCTGTGGATGATTTCTG
TCTGTGGCTAAAGCCGAAGGCTGGGCCCCACCTCCACATTTGATCGCACTCGT
GAGATTCTGCTGCTGTTGCAGTATAAACGCTAGCTATAATAGCATTTGAAAAA
AATTACAGTTCCATAAAATGCTGAAAATGAGAAATTAAACCTGCAAGTGAAAC

ATTTGAAATTAGCATACTTTATAAGATGCAGTTGGGACAAAGATGGCTTAAGT
ACTGATATTTAAGGAAAAAGTTTTCTTTCTCTTTTGGTTTATTGATTTAGTTTA
ATTTCTATTATGATATTTTGCATAATCAAGGCATTGTAAATCTTATAATTTAAA
AATAAATTACTTACGAACAGTTGTCATTGTTATGTTTTGTCATTGATTCTCATT
GCTGTCTAGTTCCTTTCTGGTATTAGCCTCTCCTTCTGTATGTTCACAGGCTCC
ATTACTGTGTTGAATTGCGTGACGTCAGGTGAGCAGTCAGGGAGGGCTGCTCT
GCGGACGCCAAGCGCACACCAGCTTGTCTCAGGCTCAGCAGTCAGCTCATCTG
GACATTTCTATTTAAAAGTCCTTTAATGTGGAAGATACACACAATTGTTACCA

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AAGGTTCTTCCAATTAATTTTACAATTTAAAAAGTATGTATTAATGTTTTATTG
TTAGATTTTCCAAAAAAAATGATGCAAATTCTGGTAATATTCATTTCCCTCACCC
ATAATTTGGTTAAAATGAGTAGTTTTAGCCATACAGTCTCATCTGCTGTGGAG
GAACCTGGAGAAAGTCCCCTGTGCCTTTCTAGCCCTTGGGTTCTATTCTTATCC
TGCAATGTCTACTGCACAGTGTGTTTGAGCAGATCCTAACCCTCCTTTTACAGT
TTCTTCTTCTTACTTCTTTATTCTTTTTGTGGCTCCTGAAATCTGAGGTTATTTT
GTAATTCAGGAGCATGCAGGACAATTGTTGGGACATGTGCCTAGTCCGGAATA
CAGCCCAGGACAGCAAGGAGATGCGTCCTGCACCAGGAAGCCGTGCAGGCAG
GAGCTGTCCAAGGTCCCGGCGGCTCTGCCTGTGTGAGGCAGGAGAATGAGCA
GATTCCCTAATCTATGTTCTCGAAGTTTAATGCTGATGTTGTCTTGCCTTATCC
TCATTTAACTGATACTGTCACCCAGTCCACCTTTGCTCTCATTGCAAAGTGATA
GTGTAATTTCAAATGTAAGACTGAAGATACGATTGTAAAAGGGAGTAAACTGG
TTTAAACGTGTTATTCTAAAGCACCTTACTTTGTTGTTGTATGCAGAAAACACA
GATGCGCTAATTCAGTATAAATGACTGATTGCCTGGAATTTGGACGTTGGCTT
AAAGTCCGATAGCTAAACCTTGGCAAAACATAACAAACATTTCATTGCTCAGC
CTCAGTGCTCTGGAGTATTCAGTGTATGAGACAGGTTTATTTGAGTCCTCTGTA
AATGGCATTTGAATTTTATATTCTCCCCTCCCGAGTATCTTATAAGACATCCCC
TGAGTTAGGGAGTTCCCAGACTGCTACTCTATTCCTTATGAATGCAAAACAAC
CACCAATAGAACAAAAAAAAAAAAAAAAACTCGAG

Mouse YNK1 partial coding cDNA - Sequence ID NO:15

CTTTACGAGCAGAGGGAGCCAAATTCAGAGCCGTTTTAGATAAAGCTGTGCAA
GCGGATGGACAGGTGAAGGAGCGCTACCAGTCCCATCGAGACACCATCGCAC
TTCTGTGTAAGCCGGAGCCAGAGCTGAATGCTGCCATCCCCTCTGCTAACCCA
GCAAAGACCATGCAGGGCAGCGAGGTTGTAAGTGTCTTAAAGTCCTTATTATC

AAATCTTGATGAAATCAAGAAGGAAAGAGAGAGTCTTGAGAATGACCTGAAG
TCAGTGAATTTTGACATGACAAGCAAGTTTTTGACAGCTCTGGCCCAAGATGG
CGTGATAAATGAGGAGGCTCTCTCTGTCACTGAGCTGGATCGGATCTATGGCG
GTCTAACAAGTAAAGTTCAAGAGTCTCTGAAGAAACAAGAGGGACTTCTAAAA
AATATACAGGTCTCACACCAAGAATTCTCCAAAATGAAGCAATCTAACAACGA
GGCTAACTTGAGAGAAGAAGTTCTGAAGAACCTAGCAACTGCGTATGACAACT
TTGTTGAGCTTGTAGCTAACTTGAAGGAGGGCACAAAGTTTTACAATGAGCTG
ACTGAGATCCTGGTCAGGTTCCAGAACAAATGCAGTGACATAGTGTTTGCACG

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GAAGACAGAAAGAGACGAGCTCTTGAAGGATCTGCAGCAGAGCATTGCCAGA
GAGCCCAGCGCTCCTTCAATCCCTCCTCCAGCCTATCAGTCCTCCCCAGCAGC
GGGGCATGCAGCAGCGCCTCCAACTCCAGCCCCAAGAACCATGCCGCCTGCTA
AGCCCCAGCCTCCAGCCCGGCCTCCACCTCCTGTGCTTCCTGCAAACCGAGTT
CCTCCTGCTTCTGCTGCTGCTGCCCCTGCAGGCGTGGGGACGGCTTCAGCAGC
GCCGCCACAGACCCCTGGCTCTGCTCCCCCGCCACAGGCTCAGGGACCACCAT
ACCCTACCTATCCAGGATATCCCGGGTATTGCCAAATGCCCATGCCCATGGGC
TACAACCCCTACGCATATGGCCAGTACAATATGCCGTACCCACCGGTGTATCA
CCAGAGCCCCGGACAGGCTCCATACCCAGGACCCCAGCAGCCTACCTACCCCT
TCCCTCAGCCCCCGCAGCAGTCCTACTATCCACAGCAGTAA

Mouse YNK1 partial protein - Sequence ID NO:16

LRAEGAKFRAVLDKAVQADGQVKERYQSHRDTIALLCKPEPELNAAIPSANPAKT
MQGSEVVSVLKSLLSNLDEIKKERESLENDLKSVNFDMTSKFLTALAQDGVINEE
ALSVTELDRIYGGLTSKVQESLKKQEGLLKNIQVSHQEFSKMKQSNNEANLREEV
LKNLATAYDNFVELVANLKEGTKFYNELTEILVRFQNKCSDIVFARKTERDELLK
DLQQSIAREPSAPSIPPPAYQSSPAAGHAAAPPTPAPRTMPPAKPQPPARPPPPVLP
ANRVPPASAAAAPAGVGTASAAPPQTPGSAPPPQAQGPPYPTYPGYPGYCQMPM
PMGYNPYAYGQYNMPYPPVYHQSPGQAPYPGPQQPTYPFPQPPQQSYYPQQ

Mouse novel cDNA clone 4: - Sequence ID NO:17

GGTCTTGGCTAGAATTTTAAATTTCTTCTCATTTGAGTAAAATGTTGCATTCTG
AAGTCCCATGCTACCTGAAGTTGCATTTGGAGTCCCAAGCTACTGGAATGTTT
ATATGTGACCGTTTCCCAGGAGGCTTACACTGCAGAAGGAAGAATGAATCTAG

GTGAGGTGGGCAGCTGCTTGGCAGTCCTCTCTTGTGCCCCAACTGTAAACCAG
ATAGAAATGTTCAGGGGAGGATACTTTCATTATTGTGGTTTGTAGTGTTAAGA
TGATTGCTTCTGCCTTGGAAATACCTCAAGCTGTTCTTATTTAACAGGTAAGTG
ACTGAGTATAATATTCCAGAAAAATTTGAAATCCTAATTTCTTCCATATTTCAT
TAAATTTTTTGCATACAGGTCTAACAAATATGGATATGTATACACATCCTCTTT
AATGAAGGTATTATTTTGGTTACTTTTCCTAAGATATACCTTAAAAGATGTTCT
ATACATTTCCTACTTAAATTCTGGGGGATTTGGAGTATGTACATGATAAAAAA
GATTATAATATATCGATTGAAGTTACTTTATTTTCTAATTAGAATTATTTTAAT
AGTCCTTTATTGAATAAGTGCTGTAATTTGTTTGCTATGAGACTTATTCCTGAT
GTGAATGTAAATTATTTTTCCACATGCATGAAAAAATGTATGTACTAATCAGA
GTTGTCTCCATTGCATTGAAATTACTTGTTTTGAACTAAAGTAACTCATATTTA
TGTAGTAGAATGCTTATGTTTTCAGACTTTGTAATGATTTCCTTTGGATGTATT
TTAAATCAATCGGTCTGGGTAACATATCAGTTTAGATTAATATGTGCTTAAAA
GAAGAAAAAAATTCAATGGTTCATAGTAGAAATGTGCCACACTTAAATAAGCT
CTGTATGACATGAAATTCTGTTAAAACATTGTAATTCATGGTGACTTTTAACTT
ATAAAAATACTACTTGCACGGGTTACTTGATTTATGGATATATGAAAACTTCTC
AGGACGAAAGTTCTTCTTTCTCTAGAACTATTCTTCTGTCGGTCATGCAGAATG
CTGTTATTCTGAAAAGTGTCCCTGTTGCATATGATGGTCACTTTATTTGGGGGG
ATTCTTCATAAGATGTGAGATGTTGATGCCAGTCTTTCCCAAGTAAGTGCTCGT
AAAAAAGGACTACTAACTAGCCTGCATCTGTCTCTAACTGGGACCAAGGGGTC
TGCTGAAGGAAACTGAAGAGCTCTAACATTTTCACAGCTTGGAGAAGATAGAA
TCTTTAAAAGTACAACTGAAGCTTGATCTATTTTACAAGTGCATTGATGGCCCC
TGTCCTTCTCTGGTTCCTGTCATTTGAAACCAACTCCTGTTGTAAATAGGAAGA
ATATGGGACATTCATATTTAAGAAAATTTGATGTCATTAGGTGACTAAGTAGA
AGGCTTAGAAAAATGTATTCATTTGCAAGTATTTTGGCACAAGAAATTTTCCA
ACTGAATAGTAAGCAAAAGCTAAGTTGTTTCATTGAAATCATAAGGCAGTTTA
AGATAAACTGGAGAAGATAACTGTTCTAATAGAGGATAATCGAATTGATTGTC
AAGTGGATGTTATTTATTGGATAGTGACAGAGTTTATTTGTAACCTTAATTATA
TTAAAAGTTATTCTGTTAGGATGTTTTGTATTAATAAACGTGAACAAAATTAA
AAAAAAAAAAAAAAAAAACTCGAGGG

Mouse Novel cDNA clone 8/47/52: - Sequence ID NO:18

GAGAAGGCGGCCTGCCGCAGCGGGACAACCTAGAGCGCGACGTGGAGGCGCG
TAGCGGAGCTGGAGCAACTGCGCACCGAGGTGGATGTGCGCATTAGCGCNNT
GGACACCTGCGTCAAGGCCAAGTCGCTGCCAGCCGTCCCGCCGAGAGTCTCAG
GCCCACCCCCGAACCCTCCACCCATTGATCCAGCTAGCCTGGAGGAATTCAAG
AAAAGGATCCTGGAGTCTCAGCGGCTCCCTGTAGTCAACCCTGCWGCCCAACC
CAGCGGTTGAGRACCCAGCTGCCGCAGGACGCTGGGTGCCAGAATCGCCCAC
CTGTGGATGGGGGCAGCCAGGTGCCCACAGTGCTGGACACCCGCCGTGCCTG
CCGGCAGCCTCCACCCCCAGCGCCTTCTCTGGCACCCCTTCACTGTCCCSTGCA
TCCCCRCCATTCSSCASWSASKGGATTTAAGGCACACACAGCTGTGAGATGACT
TCACATCGACCCCTTGTGCAGTGACCCGGATGGTGCCCCACCCACACATGAAG
CACCCACAGCTCAGCTGCCACCCTAGGCAACTCCTCCGGTTTCCTATCACTCTG
CTCCTGACCCGGGAGGTGAGAACAGGAAGCCCAGCCTTCAGCTCCCTTGGGAG
TTTCCAGCCTCCCTCTTAAAGGCCACTAGGGTTTCCAGATCCTATTTGAGAGTC
TCCAGGCCTCCCCTGAAGGGTTCTAGCCACCACGCCCACAGGATTCCCATTAG
GTTTTAAAGTCTTTTCCAGAGTCCGCTGGTTCCCCTCCTCCTCACAAGGAAGGG
CCTCAATTGTAGATGAGCGTTCCGGGTGGATCTTAGAGCCCTAGAGGGAGGCT
TTTGCTTGTARCCCCCTAAAGATATTACTGGCACATAATAAATATGAAAGTCCT
TTGAAA

GTTGGACACTGCGCAAATGGGGCTCTCCATGGACCGCAGCCCATACGCCCGCA
CGGGGGACCAGCAGCGCGGCTCTGGTTCTACCTGCGCTATTTCTTCCTCTTCGT
GTCGCTCATTCAGTTCCTCATCATCCTGGGCCTGGTCCTCTTCATGATCTATGG
CAATGTGCACGCCACCACTGAGTCCAGCCTGCGCGCCACGGAGATCCGCGCCG
ACAGCCTGTACAGCCAGGTGGTTGGACTATCGGCCTCACAGGCTAACCTGAGC
AAACAGCTGAACATCAGCTTGCTTGTCAAGGAAACAGTCATGCAGCAACTGTT
GACTACGCGACGTGAGATGGAGCGCATCAACGCCAGCTTCCGCCAGTGCCAA
GGCGACCTGATCACCTACATAAACTATAATCGCTTCATCGCCGCTATCATCCTG
AGCGAGAAGCAGTGCCAGGAACAGCTGAAGGAGGTCAACAAGACCTGCGAAC
TTTACTCTTCAAGCTGGGAGAAAAAGTTAAGACACTGGAGATGGAGGTGGCC
AAGGAGAAGGCAGTGTGCTCCAAGGACAAGGAGAGCCTGCTGGCAGGAAAGC
GGCAGACGGAAGAGCAGCTGGAGGCCTGTG

Mouse Novel cDNA clone 18/25: - Sequence ID NO:19

5'end of partial clone

TGTGCGCCGCCTCTAGAACTAGTGGATCCCCCGGGCCTGCAGGAATTCCGGCA
CGACGGCCGAGCGCCGCGGACCACCCGCGGCTGCCCGCCGAGCCGTCGACAT
GTGGGGGGACTGGGGTGGGAGCGGCCGGAGCAGCGCCAGGTACCCGGGCGC
GCAGAACCATGGCTCTCGCTCGCCTGTCCTGACCTGGCTTGCTCGCCCCACCG
AAGAATGTCAGCCAAGTCCAAGGGGAACCCTCCTCGTCCTCCGCAGCCGAGGG
ACCGCCGGCAGCCTCCAAAACCAAGGTGAAGGAGCAGATCAAGATCATAGTG
GAGGATCTGGAATTAGTCCTGGGCGACCTGAAGGACGTGGCCAAAGAACTTA
AGGAGGTGGTTGACCAGATTGACACCCTGACCTCTGATCTACAGCTGGAAGAT
GAGATGACCGACAGCTCCAAAACAGACACTCTGAACAGCAGCTCCAGTGGGA
CAACAGCCTCCAGCATAGAGAAGATCAAAGAACAGGCCAATGCTCCCCTCATT
AAACCTCCAGCACACCCGTCTGCTATCCTGACTGTCCTGAGAAAGCCAAACCC
TCCACCGCCTCCTCCAAGGTTGACACCCGTGAGGTGTGAAGAGCCTCAGAGAG
TGGTGCCGACTGCCAACCCTGTAAAGACCAATGGCACTCTTCTGCGGAATGGA
GGCTTAGCGGGGAGGCCCAACAAAATTCCAAATGGAG

3' end - Sequence ID NO:20

CTCGAGTTTTTTTTTTTTTTTTTTTTTTTTTTCATTATTTACTATTATTTATTGA
CATATTTCCAAAGCTCAAAATATTTTATTATACATATAGTTGAACATATGTTTC
AAATTGTATAGTATAGAAAATAAACTTTTTTGTAGTGTCCTCAGCATTTCATGA
TGCAAAACTATTGACAAACATCTTTAGAAAAATAATAAAATAGTCCTTCGGTA
TTAAAATTCTTATTAAAAAGCATTAGATCAAAGGGAGAACTATGACATCATCA
ATGCATAGATGAGATAGGCATGAATGGAATGAGTTGCCCTGGCTTTATCAACA
AATCAAAATATCTGACATCCCAGCTCTTATAATAGACCAAAATACTTGGAATC
AGAAGGTCACAGTTTGTTTTAGGTCAATCACAAAAAAATAAAATTCATTCATA
CTTTCTCAATTTTCCGCAGTTTCTGATGATGGAACATAGAAAACAATGTACGTC
CAGGACAGAGGCGCTACTCTGCATACTTACCACGTGATTTTTTATGCCACTTTG
TTGAATGCAGATTAATATATTTGGGCTTTTTATTGCTTGAGTAGAAAGTGCTCA
TTACTTATTATTTTACGTTTATCATATAGAAAATTAAAAACAAACAGAACGTTT
TCTTAAATGGCAGATATCACACTGTGGTAGTGGTGGATTTCCTCAGGATGGTC

TTCTGTGGTTTTGGTGCAGCGGGAGGAGGCACGGTTGCAGGTGTGGGAGGGG
GGAAACTGTTACTGTGGCTTATTCCCAGTCCCCCATTTTCTAATGGGAAAT

Mouse Novel cDNA clone 95/ EST accession #AA119951: - Sequence ID NO:21

5

GCACAGCCCCCCTCCATCCTGAAGAAAACCTCAGCGTATGGGCCTCCAGCTTC
GGGCCGTGTCTATCCTTCCTCTCCTGGGACATGGTGTTCCCCGCTTGCCCCCCT
GGCAGAAAACCG

TABLE 1

# Ese Proteins

```
Ese1  MAQFPTPFGGSLDVWAITVEERAKHDQQFLSLKPIAGFITGDQARNFFFQ
      ::::::    :    :::: ::: ::: ::  :::  : :::::::: :: :
Ese2  MAQFPTAMNGGPNMWAITSEERTKHDKQFDNLKPSGGYITGDQARTFFLQ

EH1   SGLPQPVLAQIWALADMNNDGRMDQVEFSIAMKLIKLKLQGYQLPSTLPP   100
      :::: :::: :::: : : :: ::: ::::::::::::::: :::  :::
      SGLPAPVLAEIWALSDLNKDGKMDQQEFSIAMKLIKLKLQGQQLPVVLPP   100

      VMKQQPVAISSAPA-FGIGGIASMP---PLTAVAPV--PMGSIPVVGMSP
       ::: :       : :: :          ::  :::   :  :       :
      IMKQPPMFSPLISARFGMGSMPNLSIHQPLPPVAPIATPLSSATSGTSIP

      PLVSS---VPPAAVPPLANGAPPVIQPLPAFAHPAATWPKSSSFSRSGPG   191
      ::      ::      : ::    ::::         : :  :: :    :
      PLMMPAPLVPSVSTSSLPNGTASLIQPLS-IPYSSSTLPHASSYSLMMGG   199

      SQLNTKLQKAQSF-DVASAPPAA---------------EWAVPQSSRLKY
            :::::  :  :                      :::::: :::::
      FG-GASIQKAQSLIDLGSSSSTSSTASLSGNSPKTGTSEWAVPQPSRLKY

EH2   RQLFNSHDKTMSGHLTGPQARTILMQSSLPQAQLASIWNLSDIDQDGKLT   275
      :: ::: :: ::: : : :::  : :: : : ::: :: : ::: :: :
      RQKFNSLDKGMSGYLSGFQARNALLQSNLSQTQLATIWTLADIDGDGQLK   298

      AEEFILAMHLIDVAMSGQPLPPVLPPEYIPPSFRRVRSGSGMSVISSSSV
      :::::::::: : :  :::::  ::::  :::::      :  :    ::
      AEEFILAMHLTDMAKAGQPLPLTLPPELVPPSFR-----GGKQV---DSV

      DQRLPEEPSSEDEQQPEKKLPVTFEDKKRENFERGSVELEKRRQALLEQQ   375
        ::       :  : ::::::::::   : :::  ::::::: : :::
      NGTLPSYQKTQEEE-PQKKLPVTFEDKRKANYERGNMELEKRRQVLMEQQ   389

      RKEQERLAQLERAEQERKERERQEQEAKRQLELEKQLEKQRELERQREEE
        : :: :: :  : ::: :: :::: : :::::: ::::::::::::::
      QREAERKAQKEKEEWERKQRELQEQEWKKQLELEKRLEKQRELERQREEE

      RRKEIERREAAKRELERQRQLEWERNRRQELLNQRNKEQEGTVVLKARRK   475
      :::::::::::: :::::: ::::: :::::: :   :::  : :  : :
      RRKEIERREAAKQELERQRRLEWERLRRQELLSQKTREQEDIVRLSSRKK   489

C-C   TLEFELEALNDKKHQLEGKLQDIRCRLATQRQEIESTNKSRELRIAEITH
       :  :::: : :  :  : ::::  :  ::  : :  :  : : ::
      SLHLELEAVNGKHQQISGRLQDVQIRKQTQKTELEVLDKQCDLEIMEIKQ

      LQQQLQESQQMLGRLIPEKQILSDQLKQVQQN--SLHRDSLLTLKRALEA   573
      ::: : : :  :  : :::: :    :  :          :::  :  :
      LQQELKEYQNKLIYLVPEKQLLNERIKNMQLSNTPDSGISLLHKKSS-E-   587

      KELARQQLREQLDEVERETRSKLQEIDVFNNQLKELREIHSKQQLQKQRS
      ::   : : :::: : :: ::: : : ::::::::::    :::
      KEELCQRLKEQLDALEKETASKLSEMDSFNNQLKELRESYNTQQLALEQL
```

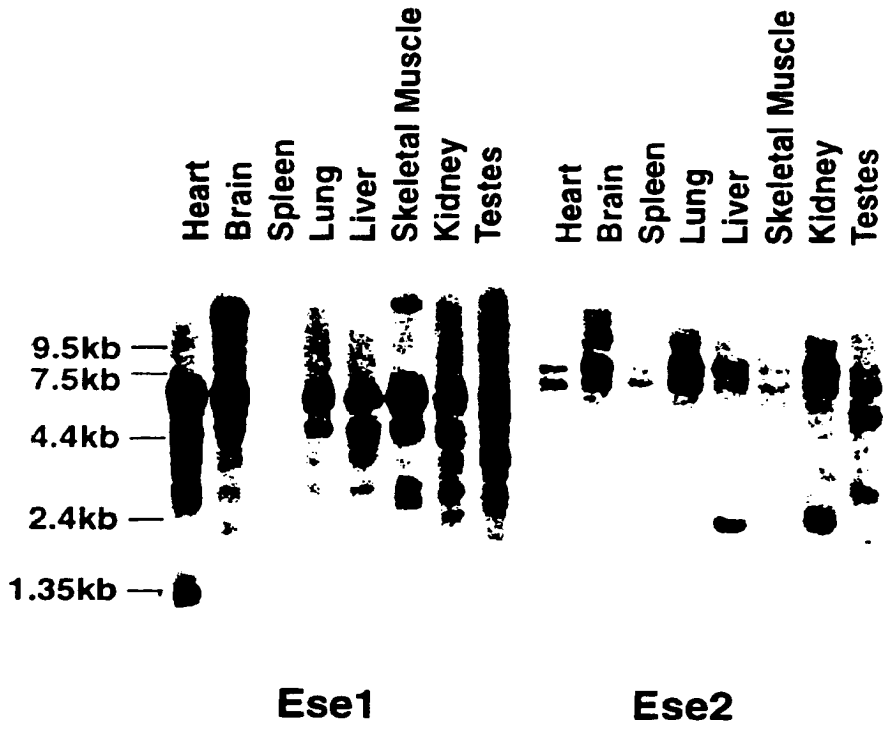
TABLE 1 Continued

```
          LEAARLKQKEQERKSLE-LEKQKED--AQRRV-QERDKQWLEHVQQEEQP   669
          HKIKRDKLKEIERKRLEQIQKKKLEDEAARKAKQGKENLWRESIRKEEEE   687

          RPRKPHEEDRLKREDSVRKKEAEERAKPEMQDKQSRLFHPHQEPAKLATQ
          KQK------RLQEEKSQDKTQEEER-KAEA--KQSET-------------

SH3A      APWSTTEKGPLTISAQESVKVVYYRALYPFESRSHDEITIQPGDIVMVDE   769
          ----------------ASALVNYRALYPFEARNHDEMSFSSGDIIQVDE   748
```

---

```
          SQTGEPGWLGGELKGKTGWFPANYAEKIPENEVPTPAKPVTDLTSAPAPK
          KTVGEPGWLYGSFQGKEGWFPCNYVEKVLSSE-----------KALSPK

          LALRETPAPLPVTSSEPSTTPNNWADFSSTWPSSSNEKPETDNWDTWAAQ   869
          KALLPPTVSLSATST-SSQPPASVTDYHNV---SFSNLTVNTTWQ-----   827

          PSLTVPSAGQLRQRSAFTPATATGSSPSPVLGQGEKVEGLQAQALYPWRA
          ------------QKSAFTRTVSPGSV-SPIHGQGQAVENLKAQALCSWTA

SH3B      KKDNHLNFNKSDVITVLEQQDMWWFGEVQGQKGWFPKSYVKLISGPVRKS   969
          KKENHLNFSKHDVITVLEQQENWWFGEVHGGRGWFPKSYVKLIPGNEVQR   914

          TSIDTGPTESPASLKRVASPAAKPAIP-GEEFIAMYTYESSEQGDLTFQQ
          GEPEALYAAVTKKPTSTAYPVTSTAYPVGEDYIALYSYSSVEPGDLTFTE

SH3C      GDVIVVTKKDGDWWTGTVGDKSGVFPSNYVRLKDSEGSGTAGKTGSLGKK  1068
          GEEILVTQKDGEWWTGSIGERTGIFPSNYVRPKDQENFGNASKSGASNKK  1014

SH3D      PEIAQVIASYAATGPEQLTLAPGQLILIRKKNPGGWWEGELQARGKKRQI
          PEIAQVTSAYAASGTEQLSLAPGQLILILKKNTSGWWQGELQARGKKRQK

          GWFPANYVKLLSPGTSKITPTELPKTAVQPAVCQVIGMYDYTAQNDDELA  1168
          GWFPASHVKLLGPSSERTMPT-------FHAVCQVIAMYDYMANNEDELN  1107

SH3E      FSKGQIINVLNKEDPDWWKGEVSGQVGLFPSNYVKLTTDMDPSQQ       1213
          FSKGQLINVMNKDDPDWWQGETNGLTGLFPSNYVKMTTDSDPSQQWCADL  1157


          QALDTMQPTERKRQGYIHELIQTEERYMDDLQLFEQKTLL             1197
```

FIGURE 1


Heart
Brain
Spleen
Lung
Liver
Skeletal Muscle
Kidney
Testes
Heart
Brain
Spleen
Lung
Liver
Skeletal Muscle
Kidney
Testes
9.5kb
7.5kb
4.4kb
2.4kb
1.35kb
Ese1
Ese2

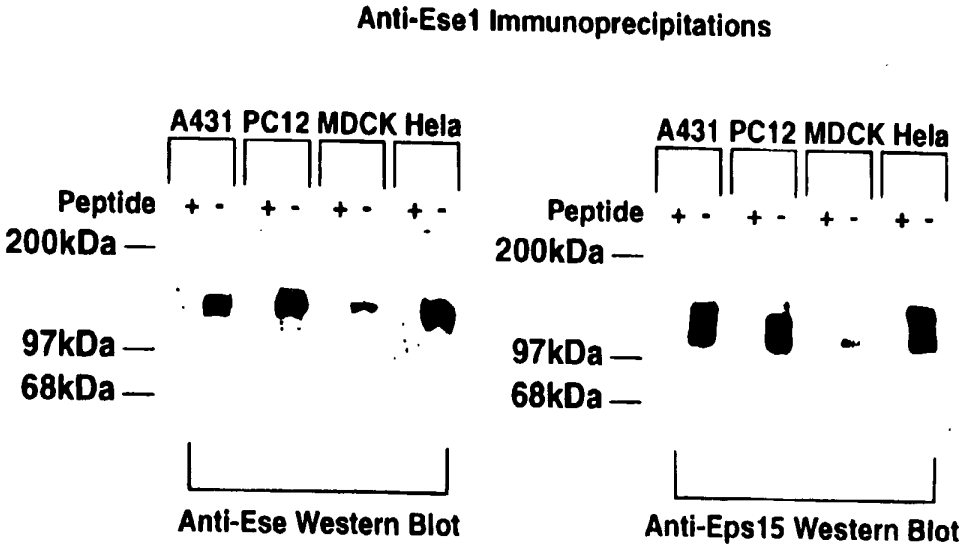



FIGURE 2

FIGURE 3

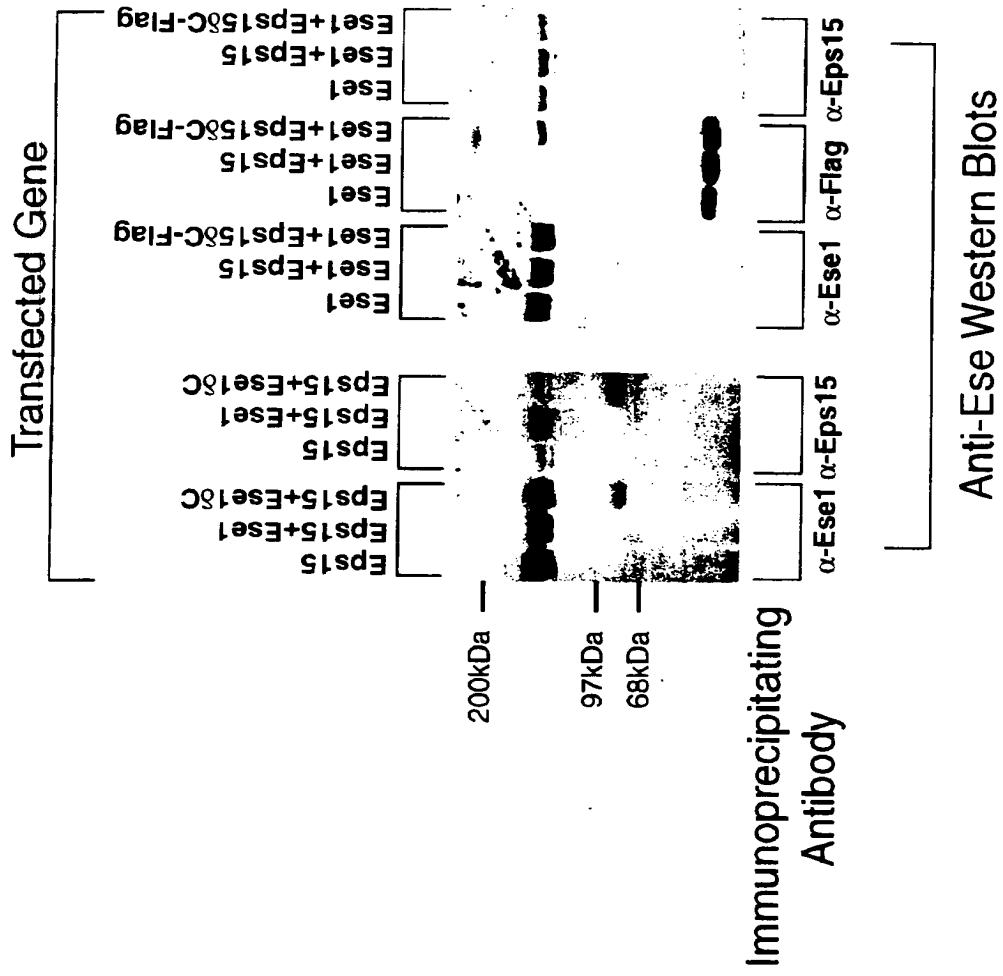

Transfected Gene
Eps15
Eps15+Ese1
Eps15+Ese1δC
Eps15
Eps15+Ese1
Eps15+Ese1δC
Ese1
Ese1+Eps15
Ese1+Eps15δC-Flag
Ese1
Ese1+Eps15
Ese1+Eps15δC-Flag
Ese1
Ese1+Eps15
Ese1+Eps15δC-Flag
200kDa
97kDa
68kDa
Immunoprecipitating Antibody
α-Ese1 α-Eps15
α-Ese1 α-Flag α-Eps15
Anti-Ese Western Blots

FIGURE 4

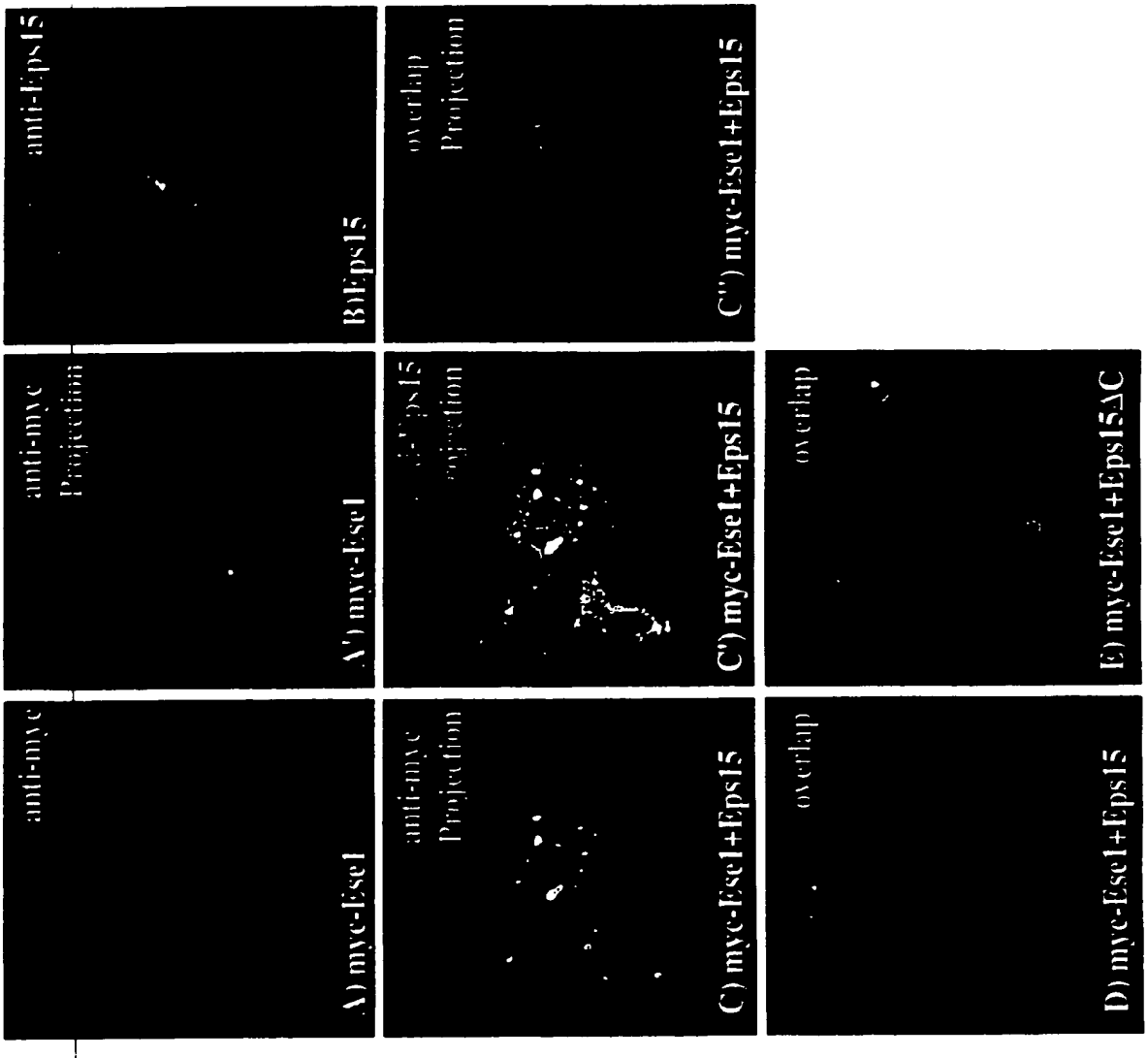
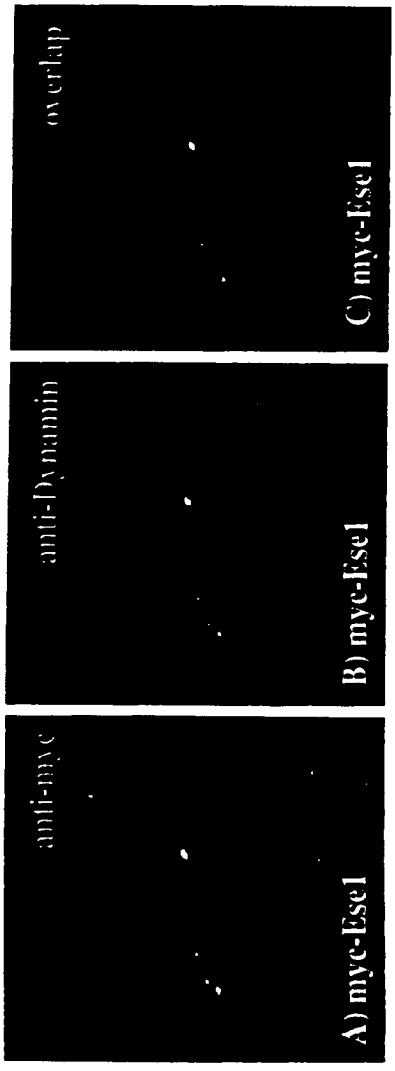

anti-myc
A) myc-Ese1
anti-myc
Projection
A') myc-Ese1
anti-Eps15
B)Eps15
anti-myc
Projection
C) myc-Ese1+Eps15
anti-Eps15
projection
C') myc-Ese1+Eps15
overlap
Projection
C'') myc-Ese1+Eps15
overlap
D) myc-Ese1+Eps15
overlap
E) myc-Ese1+Eps15ΔC

FIGURE 5



# Model: The Ese:Eps15 complex recruits critical components for coated pit formation and scission

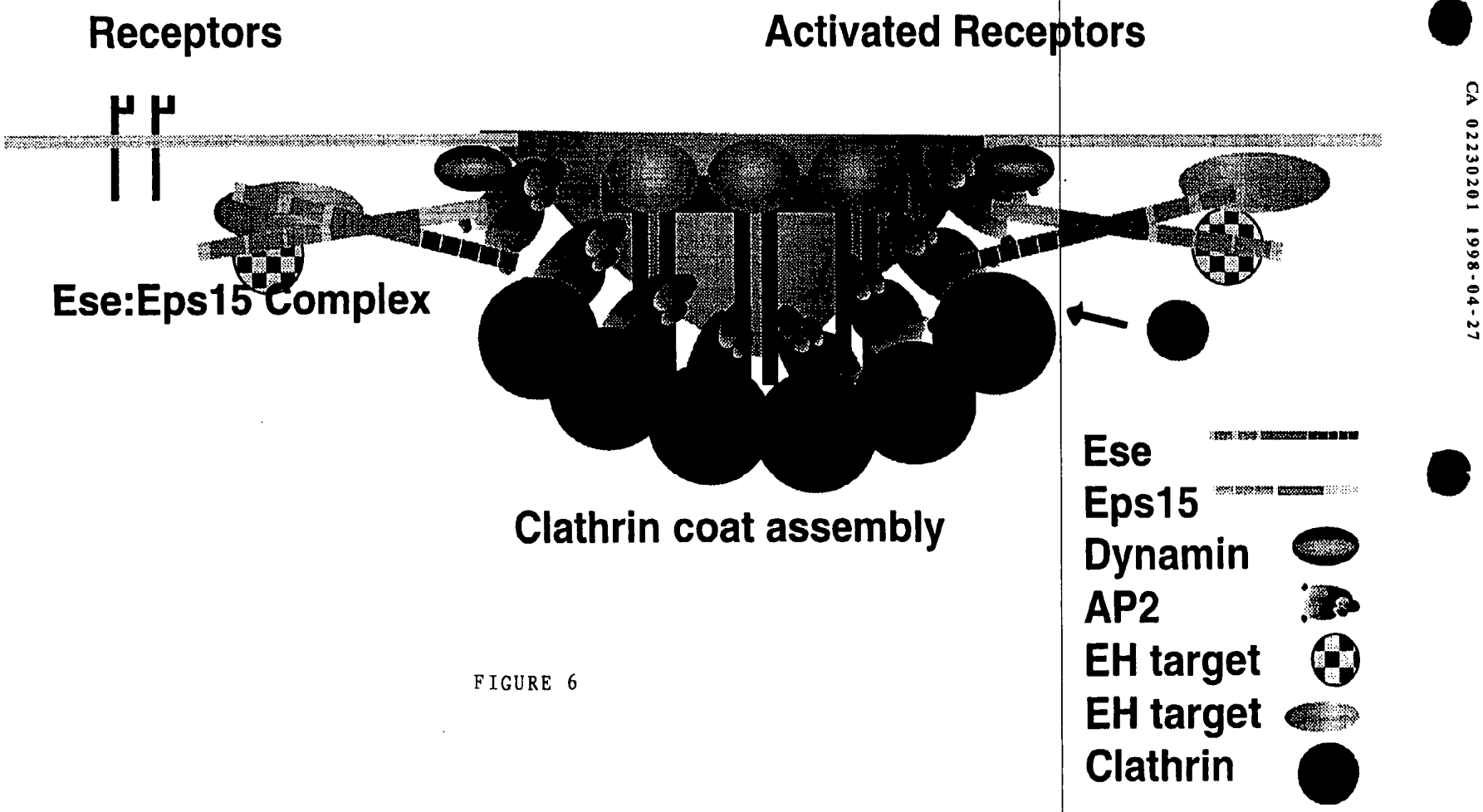



FIGURE 6